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The Secretary of Health, Education, and Welfare has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through January 31, 1980.

Sister Chromatid Exchanges in Lymphocyte Cultures of Patients Receiving Chemotherapy for Malignant Disorders [1,2]

Bo Lambert,* Ulrik Ringborg, Elaine Harper, and Anette Lindblad [3,4]

SUMMARY

The frequency of sister chromatid exchange (SCE) was studied in peripheral lymphocytes of patients receiving chemotherapy for various malignant disorders. Significantly increased SCE frequencies were recorded in six patients 2–7 weeks after CCNU treatment and in one patient 2 weeks after melphalan treatment. One patient treated with prednimustine and one treated with DTIC had SCE frequencies just within the upper limit of the control range 1–2 weeks after therapy. Normal SCE frequencies were recorded in nine patients studied at times varying from 3 days to several weeks after administration of cytostatic courses involving 5-fluorouracil, vincristine, adriamycin, cyclophosphamide, bleomycin, thioTEPA, methotrexate, and prednimustine. Radiotherapy could not be causally related to increased SCE frequencies in any of the patients. The results indicate that CCNU and melphalan, in contrast to several other chemotherapeutic agents, induce very long-lived DNA lesions, which may be of crucial importance for the development of secondary neoplasms.

[Cancer Treat Rep 62:1413–1419, 1978]

Increasing evidence suggests that secondary neoplasms may arise as a late complication of successful chemotherapy for primary malignant disorders (1–5). It is therefore important to evaluate the carcinogenic potential of cytostatics currently used as well as newly designed regimens. Several recently developed tests for genetic damage have demonstrated mutagenic properties of many carcinogenic chemicals and drugs (6). Studies of genetic damage in somatic cells of patients receiving cytostatic therapy may therefore not only improve our knowledge of the mutagenic potency of the treatment but also may provide important information for the evaluation of carcinogenic risk.

The analysis of sister chromatid exchange (SCE) is a useful technique for this purpose since it can be applied to human lymphocytes relatively easily (fig 1). SCEs arise as a consequence of breaks in the DNA molecules followed by an exchange of chromatid segments within chromosomes during the S phase of the cell cycle. Several studies have demonstrated increased SCE frequencies in cells treated with mutagenic and carcinogenic agents in vitro (7–10) and in vivo (11). Recently, Carrano et al (12) showed a linear relationship between carcinogen-induced mutation rates and SCE frequencies in cultured cells. Thus, SCE appears to be not only a sensitive indicator of DNA damage, but also a useful indicator of those specific and probably rare DNA lesions that are responsible for mutagenesis and possibly carcinogenesis.

We have studied SCEs in lymphocytes of 19 patients receiving chemotherapy for different types of neoplastic diseases. Significantly increased SCE frequencies were recorded in seven patients. In some cases, this effect was observed many weeks after drug administration.

METHODS

SCEs in PHA-stimulated lymphocytes were studied with the fluorescence plus Giemsa technique

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[3] Department of Clinical Genetics and Radiumhemmet, Karolinska Hospital, Stockholm, Sweden.

[4] We are grateful to Monica Antonsson, Kristina Blomberg, Eva Grafström, Ingrid Volny, and Barbro Werelius for technical assistance.

**Reprint requests to:* Bo Lambert, MD, Department of Clinical Genetics, Karolinska Hospital, 104 01 Stockholm 60, Sweden.

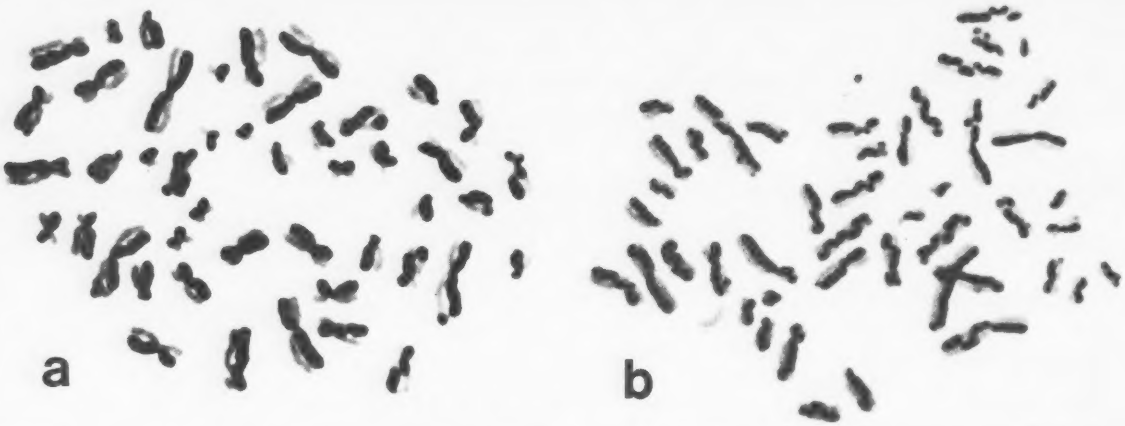

FIGURE 1.—Human lymphocyte chromosomes stained differentially to demonstrate SCEs. (*a*) Chromosome complement of a control subject showing 15 SCEs, (*b*) chromosome complement of a melanoma patient receiving CCNU chemotherapy (No. 18, table 2) showing 70 SCEs.

(13). Culture conditions, staining procedures, and SCE counting in the light microscope were as described previously (14). Bromodeoxyuridine, which is needed to label the chromatids differentially, was added at the start of the cultures at a final concentration of 100 μM. The mean value of the SCE frequency for each subject was based on the numbers of SCEs in a minimum of 20 cells. The growth of cells in cultures from some of the patients was inhibited, probably as a consequence of the cytostatic treatment, and therefore the number of cells available for analysis was not always 20 in these cases.

RESULTS AND DISCUSSION

The compiled data for the control and patient groups are shown in table 1. We have reported previously (15) that cigarette smokers have a higher average SCE frequency than nonsmokers. Therefore, the control group was subdivided into smokers ($\geq$ 1 cigarette/day) and nonsmokers. The difference in SCE frequency between the two control groups is statistically significant (15). In agreement with others (16), we have not found age or sex to influence the SCE frequency significantly among adult control subjects. Cells with high numbers of SCEs are rare among control subjects and are mostly found in smokers. Of > 1000 cells analyzed in the control group, only 11 have $\geq$ 30 SCEs (table 1).

The average SCE frequency in the patient group is higher than that of the nonsmoking as well as the smoking control groups (table 1). Clinical data and SCE scores for the individual patients are compiled in table 2. In the evaluation of the individual SCE frequencies we have considered a value of two standard deviations (SD) above the average SCE frequency of the corresponding control group as an increased value; this is 18.9 SCEs/cell for nonsmokers and 23.4 SCEs/cell for smokers. All patients with an increased SCE frequency according to this definition have a large fraction of cells with $\geq$ 30 SCEs. Cells with such high numbers of SCEs are rare among patients with an average SCE frequency within the control range (table 2). Of seven patients demonstrating increased SCE frequencies, six have been treated with CCNU (Nos. 8, 10, and 16–19) and one has been treated with melphalan (No. 14). In three additional patients (Nos. 5, 12, and 15) there is a small fraction of cells with $\geq$ 30 SCEs but the average SCE frequency in these patients is just below the upper limit of the control range as defined above.

The SCE analyses were usually made midway between cytostatic courses or immediately before the next course. Thus, the time between treatment and analysis differs in various patients from < 1 week to > 2 months. In some patients (Nos. 3, 6, 12, and 15–17), a second analysis was made after a prolonged therapy-free period or after additional courses. Therefore, the individual treatment schedules need a more detailed consideration in order to evaluate the possible influence of radiotherapy and chemotherapy on the SCE frequency.

Radiotherapy was given to 12 of the 19 patients. In all cases, full tumor doses were given. Several patients (Nos. 1–4, 6, 11, and 13) still demonstrated SCE frequencies within the normal range. Among

TABLE 1.—SCEs in controls and patients: compiled data

Group of subjects	No. of subjects	Age in yrs		Sex (M/F)	Average No. of SCEs/cell/individual		No. of SCEs/cell		
		Mean	Range		Group means ± SD	Range	No. of cells analyzed	Range	No. of cells with ≥ 30 SCEs
Controls*									
Nonsmokers	29	34	19–56	26/6	13.1 ± 2.9	7.8–19.6	678	3–34	1
Smokers	14	40	20–66	7/7	16.2 ± 3.6	9.2–24.2	388	2–48	10
Patients†	19	56	18–80	13/6	22.7 ± 11.1	6.0–48.1	461	3–95	95

*Controls were occupationally nonexposed, healthy subjects who were analyzed during the same time period and by the same technique as the patients. 5 controls were students drafted for military service, 8 were medical students, 7 were employees mainly occupied as clerks or salespersons, and 22 were regular blood donors of various professions. Smokers are defined as regular smokers of ≥ 1 cigarette/day.

†Data for the individual patients are given in table 2.

these patients, Nos. 3 and 13 received radiotherapy during the 6 months preceding the SCE analysis. Patient 6 received 4000 rads 1 month prior to the first analysis (No. 6a, table 2) and showed an SCE frequency within the normal range. Four months later and after sustained methotrexate therapy, the SCE frequency in this patient was very low compared to smoking controls (No. 6b, table 2). These results indicate that radiotherapy does not cause a long-lasting increase in the frequency of SCE, although such an effect of short duration immediately after irradiation cannot be excluded.

Actinomycin D (patient 13) and 5-fluorouracil (5-FU) (patient 1) did not cause a significant increase in the frequency of SCEs although they were given as late as 9 and 4 days, respectively, before the SCE analysis.

Vincristine (VCR) and vinblastine were not given as single drugs to any of the patients, and their effects are difficult to evaluate. Low SCE frequencies were recorded in patients 2 and 11 who received vincristine 1 and 4 months, respectively, before SCE analysis.

Bleomycin and thioTEPA were given to patient 4, who also received 5-FU 6 weeks earlier. ThioTEPA was given as an intrapleural infusion 10 days before SCE analysis and bleomycin as an im injection 3 days before. No increase in the frequency of SCEs was recorded in this patient.

Methotrexate was given to several patients, but in most cases in combination with other drugs. Patient 9 received very large doses (15 g) weekly during 3 months and yet showed an SCE frequency well within the normal range 1 week after the last injection. Patient 6 had a normal SCE frequency 1 week after a single administration of methotrexate (No. 6a, table 2). Four months later, after repeated weekly administrations of methotrexate, the frequency of SCEs in this patient was much lower (No. 6b, table 2). The earlier analysis in this patient was made shortly after radiotherapy and it is possible that the high SCE value at the first analysis was a consequence of the radiotherapy as such or of a synergistic effect of the radiation damage and chemotherapy.

Adriamycin was given in repeated courses in combination with 5-FU and VCR to patient 2 who, 1 month after the sixth course, demonstrated a very low SCE frequency. Patient 8 showed an increased SCE frequency 1 day after receiving 30 mg of adriamycin, but in this patient it is not possible to distinguish the effect from that of CCNU (see below). In patient 10 the adriamycin course was discontinued 6 months before SCE analysis, and the high SCE frequency in this patient was therefore probably also caused by CCNU (see below).

Prednimustine was used in combination with other drugs in three patients. Patient 5 demonstrated a high but not definitely increased SCE frequency 6 days after a 10-day course of prednimustine. The low SCE frequency in patient 11 was recorded 4 months after the last treatment with prednimustine. Patient 12 showed a low SCE frequency 2 months after receiving prednimustine (No. 12b, table 2). The earlier analysis of this patient (No. 12a, table 2) was done 5 days after prednimustine and 2 weeks after CCNU administration. The relatively high SCE frequency in this patient at the earlier date could therefore be an effect of CCNU as well (see below).

Cyclophosphamide was the only treatment in patient 7, who received 1 g daily for 4 days. One month later, this patient had an SCE frequency within the control range. Repeated courses of relatively small

TABLE 2.—SCEs in patients receiving chemotherapy: individual patient data

					Treatment†		SCEs			
Patient No.	Age in yrs	Sex	Smoking status*	Diagnosis	Earlier‡	Actual‡	No. of cells	Mean ± SD	Range	No. of cells with ≥ 30 SCEs
1	62	F	S	Metastases of unknown primary tumor	RT (4000 rads)	5-FU	20	14.4 ± 1.6	12–17	0
2	78	M	NS	Carcinoma of urinary bladder, lung metastases	RT (8400 rads)	5-FU-VCR-AM	20	6.0 ± 2.6	3–13	0
3	56	F	NS	Mammary carcinoma	RT (4000 rads)	5-FU-MTX-CPM	(*a*) 20 (*b*) 20	13.4 ± 4.8 13.9 ± 5.7	7–22 6–24	0 0
4	64	F	NS	Carcinoma of the palate, lung metastases	RT (6600 rads)	5-FU-TT-BLM	20	12.0 ± 3.2	6–18	0
5	34	M	NS	Maxillar carcinoma	RT (6400 rads)	MTX-VCR-PM	20	18.8 ± 6.3	7–32	1
6	66	M	S	Carcinoma of epipharynx	RT (6400 rads)	RT (4000 rads); MTX	(*a*) 20 (*b*) 10	17.7 ± 5.1 10.2 ± 2.3	10–29 7–14	0 0
7	69	M	S	Carcinoma of epipharynx	—	CPM	22	20.3 ± 5.4	11–29	0
8	80	M	NS	Carcinoma of rectum	RT (6400 rads); 5-FU	BLM-VCR-AM-CCNU	21	30.4 ± 10.2	13–54	10
9	18	M	NS	Osteogenic sarcoma, lung metastases	Interferon	MTX	20	16.1 ± 4.1	10–23	0
10	39	M	NS	Soft tissue sarcoma, lung metastases	RT (4000 rads); AM; VBL; MTX	DTIC-CCNU-VCR	44	44.5 ± 14.4	10–66	36
11	70	M	S	Lung carcinoma	RT (4000 rads)	MTX-VCR-PM	20	11.2 ± 4.4	3–19	0
12	69	M	S	Lung carcinoma	RT (3000 rads); BLM	MTX-VCR-CCNU-PM	(*a*) 4 (*b*) 11	23.3 ± 14.4 11.9 ± 9.2	13–44 4–32	1 1
13	55	F	NS	Melanoma	RT (4000 rads)	ACT D	20	15.0 ± 4.4	8–25	0
14	46	M	NS	Melanoma	—	MP	20	24.8 ± 7.7	6–40	6
15	51	F	S	Melanoma	—	DTIC	(*a*) 25 (*b*) 37	22.4 ± 6.6 8.5 ± 2.9	10–38 3–15	2 0
16	65	F	S	Melanoma	—	CCNU	(*a*) 16 (*b*) 5	30.4 ± 7.9 42.4 ± 32.0	10–40 14–95	10 2
17	56	M	NS	Melanoma	RT (4000 rads)	CCNU	(*a*) 20 (*b*) 7	25.5 ± 11.8 29.3 ± 13.9	7–53 17–58	8 3
18	51	M	S	Melanoma	—	CCNU	16	48.1 ± 20.2	13–72	13
19	32	M	NS	Melanoma	—	CCNU-BLM	3	37.7 ± 25.8	10–61	2

*S = regular smoker of ≥ 1 cigarette/day; NS = nonsmoker.

†*RT* = radiotherapy; *5-FU:* 750 mg/wk iv injection (patients 1 and 4); *TT* = thioTEPA: 20 mg for intrapleural infusion; *BLM* = bleomycin: 7.5 mg im twice a wk (patients 4 and 12); *MTX* = methotrexate: 250 mg/m^2 of body surface area as iv injection with citrovorum factor rescue (patient 6) or 250 mg/kg iv infusion with citrovorum factor rescue (patient 9) once a wk; *CPM* = cyclophosphamide: 1 g/day × 4 days every 4 wks; *PM* = prednimustine: 100 mg orally × 10 days every 4 wks; *ACT D* = actinomycin D: 0.5 mg iv injection × 4 days every 4 wks; *MP* = melphalan: 1 mg/kg iv infusion every 4 wks; *DTIC:* 250 mg/m^2 of body surface area iv injection × 5 days every 4 wks; *CCNU:* 130 mg/m^2 of body surface area orally every 4 wks; *VBL* = vinblastine: 2 mg iv injection every wk. *5-FU-VCR-AM:* 1 g iv of 5-FU, 2 mg iv of VCR, and 50 mg iv of AM every 4 wks; *5-FU-MTX-CPM:* 900 mg of 5-FU and 50 mg of MTX iv on Days 1 and 8 and 75 mg orally of CPM on Days 1–14 (course was repeated every 4 wks); *MTX-VCR-PM:* 100 mg/m^2 of MTX with citrovorum factor rescue and 1 mg/m^2 of VCR iv on Day 1 and 100 mg orally of PM on Days 1–10 (course was repeated every 6 wks); *BLM-VCR-AM-CCNU:* 7.5 mg im of BLM, 1 mg iv of VCR, 30 mg iv of AM every 2 wks, and 120 mg/m^2 orally of CCNU every 4 wks; *DTIC-CCNU-VCR:* 200 mg/m^2 iv of DTIC on Days 1–3 and 130 mg/m^2 orally of CCNU on Day 1 every 6 wks and 1 mg of VCR iv on Day 1 every wk; *MTX-VCR-CCNU:* 100 mg/m^2 iv of MTX, 1 mg/m^2 iv of VCR, and 80 mg/m^2 orally of CCNU every 6 wks; and *CCNU-BLM:* 200 mg of CCNU orally every 6 wks and 5 mg of BLM im twice a wk.

‡*Earlier* includes treatment up to 2 mos before analysis; *actual* includes treatment during the 2 mos before analysis.

doses of cyclophosphamide (75 mg/day × 14 days) were given to patient 3 in combination with 5-FU and methotrexate. The two analyses in this patient (No. 3a and b, table 2) were made 1 month after the second and fifth courses, respectively. No increase in the SCE frequency was recorded. Therefore, it appears likely that cyclophosphamide does not cause a persistent increase in the SCE frequency in vivo.

DTIC was used as the only treatment in one patient. The first analysis in this patient (No. 15a, table 2) was made 2 weeks after the first course and showed a high SCE frequency just below the control range for smokers. The second analysis, showing a very low SCE frequency, was made 4 weeks after the second course. Such a substantial decrease in the SCE frequency between two analyses was noted in two other patients (Nos. 6 and 12) and could reflect alterations in the cell populations caused by the treatment. The very high SCE frequency in patient 10, who received DTIC in combination with CCNU and VCR, is probably mainly a result of the CCNU treatment (see below).

Melphalan was used only in patient 14 (table 2). Limb perfusion (85 mg) was given twice, 1 year and 6 months, respectively, before two courses of systemic treatment (75 mg once a month). The analysis was made 2 weeks after the second systemic course and showed an increased SCE frequency, almost twice as high as the average control value for nonsmokers. Six of 20 cells analyzed from this patient had > 30 SCEs (table 2), compared to only one such cell among 678 control cells (table 1).

CCNU appears to be a potent inducer of SCEs in vivo as evidenced by the fact that six of seven patients receiving this drug had at least a twofold increase in SCE frequencies (Nos. 8, 10, and 16–19; table 2) compared to average control values (table 1). Patients 16–18 received CCNU as the only chemotherapy. Analyses in these patients were made 2–7 weeks after drug administration (No. 16a, 6 weeks after first course; No. 16b, 2 weeks after second course; No. 17a, 3 weeks after second course; No. 17b, 2 weeks after seventh course; No. 18, 7 weeks after second course; and No. 19, 10 days after second course). The increased SCE frequencies in patients 8 and 10 were recorded 2 and 3 weeks, respectively, after administration of CCNU in combination with other drugs. A synergistic effect of adriamycin and DTIC on the SCE-inducing property of CCNU can therefore not be excluded. In all CCNU-treated patients there was a substantial fraction of cells with ≥ 30 SCEs (table 2).

These results show that patients treated with CCNU and melphalan have an increased frequency of SCEs several weeks after drug administration. Patients treated with 5-FU, VCR, methotrexate, bleomycin, actinomycin D, adriamycin, and cyclophosphamide demonstrated SCE frequencies within the control range. In two other patients treated with prednimustine and DTIC, high SCE frequencies, though just below the upper control range, were recorded 1–2 weeks after drug administration.

Among the chemotherapeutic agents which did not cause an apparent increase of SCEs in this report, VCR (17) and bleomycin (7) induced SCEs in vitro in human lymphocytes and CHO cells, respectively. Adriamycin (18) and cyclophosphamide (17) have recently been shown to cause an increased SCE frequency in human lymphocyte cultures after in vivo treatment of cancer patients. Raposa (17) describes two patients treated with cyclophosphamide who during or immediately after treatment showed an increased SCE level. About 2–3 weeks after cessation of therapy the SCE frequency had returned to the control level. Nevstad (18) has studied a patient who received 125 mg of adriamycin in a single dose. The SCE frequency increased 10 minutes after injection, decreased gradually at 6 and 24 hours, and was at the control level after 1 month.

One reason for differences in the time course of the SCE response to chemotherapeutic agents could be the differences in the rate and extent by which DNA lesions are repaired. The likely explanation for our negative findings in patients receiving VCR, bleomycin, adriamycin, cyclophosphamide, and possibly also prednimustine and DTIC is that SCE-inducing DNA damage caused by these agents in vivo is of a relatively short duration (< 2–3 weeks), as shown for adriamycin and cyclophosphamide (17,18). In contrast, CCNU and possibly melphalan may give rise to DNA lesions of a type that is not easily removed by cellular repair enzymes. Such very long-lived DNA lesions could be of crucial importance for mutagenesis and the development of secondary neoplasms.

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Phase I Study of Methanesulfonamide, *N*-[4-(9-acridinylamino)-3-methoxyphenyl]- (*m*-AMSA) Using a Single-Dose Schedule[1]

Daniel D. Von Hoff,* Dianne Howser, Paul Gormley, Richard A. Bender, Daniel Glaubiger, Arthur S. Levine, and Robert C. Young[2,3]

SUMMARY

Methanesulfonamide, *N*-[4-(9-acridinylamino)-3-methoxyphenyl]- (NSC-249992), an acridine derivative with significant antitumor activity in animal tumor systems, was administered to 29 patients in a phase I clinical trial. The dose ranged from 10 to 160 mg/m^2 with a single dose given every 28 days. The toxic effects included moderate to severe leukopenia and mild thrombocytopenia. Myelosuppression was more severe in patients with prior whole abdominal or pelvic radiotherapy. Superficial phlebitis occurred when the drug was diluted in a volume of < 500 ml of 5% dextrose in water. Antitumor activity was detected in one patient with ovarian carcinoma. Phase II studies are indicated with this compound since it has reproducible and reversible toxicity with some evidence of antitumor activity. The starting dose of the drug for phase II trials should be 120 mg/m^2 as a single iv dose repeated at 4-week intervals.

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In 1933, Mellanby reported that acriflavine, an acridine dye, possessed antitumor activity (1). Since that time, numerous modifications of the acridine nucleus have been carried out in an attempt to increase the compound's therapeutic index (2–4). The drug, methanesulfonamide, *N*-[4-(9-acridinylamino)-3-methoxyphenyl]- (*m*-AMSA) (fig 1), is one of many acridine derivatives prepared by Cain and Atwell (4).

m-AMSA is active against a wide spectrum of mouse tumors. It has good activity in ip L1210 leukemia with a median survival time of treated over control animals (T/C) of 180%–290% (4).[4] Schedule dependency in this system has been reported by some investigators (4) but has not been seen in other studies.[4] The drug is active in ip P388 leukemia with the best T/C being 354%–541%.[4] *m*-AMSA has demonstrated good antitumor effects in B16 melanoma (best T/C, 217%–243%) where long-term survivors (> 45 days) were consistently observed (4).[4] Antitumor activity has also been noted in spontaneous C3H mammary adenocarcinoma (4), $CD8F_1$ mammary tumors (sc),[4] and the transplantable colon tumors in mice (ip No. 26 colon and sc No. 38 colon).[4] *m*-AMSA has not demonstrated antitumor efficacy in other studies of transplantable No. 38 or 36 colon tumors in mice (5). In addition, it is considered inactive in ic inoculated L1210 (4), the ependymoblastoma mouse model,[4] the MX1 breast xenograft, or the CX2 and other colon xenografts in nude mice (6).[4]

The mechanism of action of *m*-AMSA has not been entirely elucidated. The compound does affect DNA synthesis in L1210 cells in mice (7). In in vitro systems it removes and reverses the supercoiling of DNA which is consistent with the hypothesis that *m*-AMSA binds to DNA through intercalation (8).

In addition to its antitumor effects, *m*-AMSA has documented antiviral activity (9). It is also immunosuppressive in mice when injected 28 hours after an antigen (10).

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[1] Received Aug 7, 1978; accepted Sept 12, 1978.

[2] Division of Cancer Treatment (DCT), National Cancer Institute (NCI), Bethesda, Md.

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[4] Screening data from the Drug Evaluation Branch, Developmental Therapeutics Program, DCT, NCI.

**Reprint requests to:* Daniel D. Von Hoff, MD, Clinical Oncology Program, DCT, NCI, Bldg 10, Rm 3B06, National Institutes of Health, Bethesda, Md 20014.

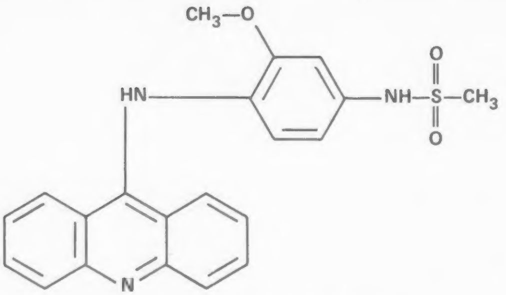

FIGURE 1.—Chemical structure of *m*-AMSA.

Toxicology studies of *m*-AMSA in beagle dogs and rhesus monkeys showed that the dog was the more sensitive of the two animal species. The toxic-dose low for the beagle dog on a single-dose schedule was 31.2 mg/m^2.[5] In dogs and monkeys, the most frequently observed toxic effects were related to the liver and to the gastrointestinal, lymphatic, and hematopoietic systems. The liver abnormalities seen at higher doses included elevated alkaline phosphatase, SGOT, SGPT, and bilirubin levels. Histologic alterations included cytoplasmic vacuolization, congestion, hemosiderosis, and hemorrhage. The gastrointestinal toxicity was manifested by anorexia, emesis, and bloody diarrhea. There was congestion, hemorrhage, degeneration, and necrosis of intestinal mucous membranes. Lymphatic tissues showed hemorrhage, congestion, and necrosis.[5]

Anemia and leukopenia were noted in peripheral blood and were dose-related. However, thrombocytopenia was not a prominent manifestation of toxicity in the dogs and monkeys. A consistent effect was noted in the animals' bone marrow with observed hypoplasia being dose-related and reversible. Other side effects included local tissue reactions at injection sites. In the dog, reversible congestion and hemorrhage in the kidneys were noted with transient increases in BUN and serum creatinine levels and with hematuria and proteinuria.[5]

A preclinical pharmacology study of the drug done in mice and rats showed that ^{14}C-*m*-AMSA was well distributed in all tissues except the brain. High levels were attained in the mouse liver, kidneys, and spleen (11). The initial plasma half-life for unchanged *m*-AMSA was 28 minutes in mice. More than 50% of the administered radioactivity was excreted in the bile within 2 hours after drug administration. The bile:plasma ratios of radioactivity were $>400{:}1$. Less than 1.5% of the biliary radioactivity was unchanged *m*-AMSA. Eleven percent of the administered radioactivity was present in the urine by 24 hours with approximately 22% of it being unchanged *m*-AMSA (11). Two groups of investigators have shown that nucleophilic attack at the acridine C_9 position by thiols is important in the metabolism of *m*-AMSA (11–13).

m-AMSA was selected for a phase I clinical trial in patients with advanced malignancies for the following reasons:

(*a*) it has antitumor activity in animal tumor systems;
(*b*) it represents a new class of antitumor agents not yet tested in man;
(*c*) it concentrates in livers of rodents, a property of theoretic importance in treating human malignancies which commonly metastasize to the liver; and
(*d*) it binds to melanin granules in the B16 melanoma system (14) which suggests the possible use of *m*-AMSA in human melanoma.

The single-dose drug schedule was selected because animal toxicity data were available for that schedule, because no clear schedule dependency was demonstrated consistently in mouse tumor systems, and because the single dose would facilitate pharmacokinetic studies.

MATERIALS AND METHODS

Studies with *m*-AMSA were initiated at the NCI in November 1977.

Patient Selection

All patients entered in the study had histologic confirmation of a malignancy refractory to standard treatment (*see* table 1). Only patients who had an estimated survival of >6 weeks were eligible. All patients had received multiple chemotherapeutic agents (an average of five different drugs) and 65% had received prior radiotherapy to the primary tumor or its metastases. All patients had measurable disease at the time of entry in this study.

Prior to therapy, all patients had adequate blood cell counts (wbcs ≥ 2500/mm^3 and platelets $\geq 100{,}000$/mm^3) except for the five patients with acute leukemia. All patients had normal renal function (BUN <25 mg/100 ml and serum creatinine <1.2 mg/100 ml). Five patients had minor elevations in SGPT and SGOT levels (<200 IU/100 ml), and three patients had elevated alkaline phosphatase levels (<400 IU/100 ml). These abnormalities appeared to be related to liver or bony metastases.

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[5] Henry MC, Port CD, Guarino AM, et al. Preclinical toxicologic evaluation of 4′-(9-acridinylamino)methanesulphon-*m*-anisidide monochloride in mice, dogs and monkeys. Report No. IITRI-Tox. 249992-76-2, NCI, National Institutes of Health, Oct 20, 1976.

TABLE 1.—Phase I m-AMSA study: patient characteristics

Characteristics	
Total No. of patients	29
Men/women	16/13
Age in yrs	
Median	42
Range	7–59*
No. of patients with—	
Prior treatment	29
Chemotherapy	29
Radiotherapy and chemotherapy	17
Tumor type	
Melanoma	8
Ovarian carcinoma	5
Sarcoma	4
Breast carcinoma	3
Acute lymphocytic leukemia	3
Acute undifferentiated leukemia	2
Colon carcinoma	1
Lymphoma	1
Testicular carcinoma	1
Nasopharyngeal carcinoma	1

*3 patients in the study population were children ≤ 14 yrs of age; all had acute lymphocytic leukemia.

Prior to entry, informed consent was obtained from the patient in accordance with federal and institutional policies.

Treatment Plan

m-AMSA is formulated as two sterile liquids that are aseptically combined prior to use. Each 2-ml flint ampule contains 1.5 ml of a 50-mg/ml solution of m-AMSA in anhydrous *N,N*-dimethylacetamide. Each 20-ml amber vial contains 13.5 ml of 0.0353 M L-lactic acid. The flint ampule (1.5 ml) containing 75 mg of m-AMSA is added to the vial of L-lactic acid solution and the contents are mixed. The resulting orange-red solution contains 5 mg/ml of m-AMSA. Initially this solution was further diluted with 150 ml of 5% dextrose in water and infused through a newly started well-running iv line. Because of phlebitis occurring at doses of ≥ 70 mg/m^2, the drug had to be diluted in 500 ml of 5% dextrose in water and infused over 1 hour. The schedule of drug administration was a single dose every 28 days.

The starting dose of 10 mg/m^2 was determined by taking one third of the toxic-dose low in the most sensitive animal species, the dog (15). Dose levels were increased as noted in table 2 with a minimum of three patients being treated at each dose level. If no toxicity was observed at a particular dose level after 3 weeks, the next dose level was started. No dose escalations were performed in the same patient in an effort to define whether cumulative toxicity would occur with monthly doses of m-AMSA.

Study Parameters

During treatment, the patients had cbc counts with differential and platelet counts, SMA-12 chemistry panels, and urinalyses twice per week. Other laboratory and radiologic examinations pertinent to tumor evaluations as well as electrocardiograms were performed prior to every course of treatment. Patients were followed closely for any evidence of toxicity and for other biologic effects. Patients were considered evaluable for toxicity if they received at least one dose of m-AMSA with at least 3 weeks of followup. Standard criteria of response were used to evaluate the antitumor effect of the drug.

TABLE 2.—Myelosuppression by m-AMSA at each dose level

Dose (mg/m^2)	No. of patients/ No. of courses	No. of courses evaluable for myelosuppression	Median (cells × 10^3/mm^3)		Median time to nadir/recovery (in days)	
			Wbc nadir (range)	Platelet nadir (range)	Wbcs	Platelets
10	3/3	1	5.4 (—)	211 (—)	—	—
20	3/4	4	4.4 (3.1–6.1)	210 (200–290)	—	—
33	3/3	1	3.3 (—)	175 (—)	—	—
50	3/4	4	4.9 (4.8–6.0)	260 (260–390)*	—	—
70	3/6	6	2.9 (2.2–4.5)	220 (190–250)	15/28	14/25
90	4/5	4	2.7 (1.8–4.3)	140 (120–390)	10/27	13/23
120	8/11	10	3.0 (0.5–4.9)	210 (15–325)	10/25	13/24
160	2/3	3	1.4 (1.2–1.6)	155 (90–200)	10/25	11/24

*Platelet counts increased in some patients.

Termination of Study

Individual patients were taken off the study if objective tumor progression occurred following one or more courses of *m*-AMSA. The phase I study was terminated when the maximally tolerated dose was established and when the cumulative toxicity of *m*-AMSA was defined.

RESULTS

Over an 8-month period, 29 patients were entered in the study. All patients were evaluable for toxicity. Patient characteristics are shown in table 1 with a listing of the types of tumors the patients had. The sequences of doses and toxic effects observed at each dose level are shown in table 2.

Myelosuppressive Toxicity

Leukopenia (total wbcs $< 3000/\text{mm}^3$) was the major dose-limiting toxic effect of *m*-AMSA (table 2). A total of 33 courses administered to 24 patients were evaluable for myelosuppression. As noted in table 2, the degree of leukopenia was related to the dose of *m*-AMSA administered. The ranges for this leukopenia are also noted in table 2. The median time to occurrence of the wbc count nadir was 10 days, with full recovery by Day 25. A dose of 120 mg/m^2 provides reproducible and reversible leukopenia in heavily previously treated patients. The two patients who had the most severe leukopenia (total wbc count nadir of 500 and $600/\text{mm}^3$, respectively) had had extensive prior radiotherapy to the whole abdomen and pelvis.

Thrombocytopenia (platelet counts of $< 100{,}000/\text{mm}^3$) was also noted. With the exception of two patients who had previously received extensive radiotherapy to the abdomen and pelvis and had a platelet count nadir of 15,000 and $28{,}000/\text{mm}^3$, respectively, the thrombocytopenia was mild (lowest counts $\geq 90{,}000$ platelets/mm^3). The median day of the platelet count nadir was Day 12 with full recovery by Day 24.

Overall, there were ten patients who received more than a single dose of *m*-AMSA (seven patients received two courses [20–160 mg/m^2], two received three courses [120 mg/m^2], and one received four courses [120 mg/m^2]). Cumulative myelosuppression was not seen in any of these patients. The hemolysis noted in beagle dogs secondary to *m*-AMSA did not occur in patients receiving the drug.

Nonmyelosuppressive Toxicity

All of the 39 courses administered were evaluable for nonmyelosuppressive toxicity. When doses of ≥ 70 mg/m^2 were infused in 150 ml of 5% dextrose in water in two patients, erythema developed immediately over the course of the vein. This erythema changed to a moderately severe phlebitis which resolved within 2 weeks. The phlebitis was prevented at higher doses by diluting the *m*-AMSA in 500 ml of 5% dextrose in water and infusing the solution over 1 hour. There were no episodes of drug extravasation during the study.

Only one patient developed nausea which was thought to be secondary to *m*-AMSA. This patient was at the 70-mg/m^2 dose level. No patients had drug-induced vomiting.

One patient at the 50-mg/m^2 dose level developed a generalized pruritic macular skin rash 1 day after drug administration. The rash resolved spontaneously within 2 days. Progressive tumor precluded a second administration of *m*-AMSA in this patient.

Two patients at the 160-mg/m^2 dose level developed mild elevations (30% increase) in serum alkaline phosphatase. This effect appeared to be drug-related, occurred by Day 10, and was reversible by Day 15. There were no other abnormalities in liver function tests. No renal, cardiac, or mucous membrane toxicity was noted in this study.

Antitumor Activity

One 39-year-old patient with ovarian cancer did experience some antitumor effects from *m*-AMSA. Her prior treatment included hexamethylmelamine, cyclophosphamide, 5-fluorouracil, *cis*-dichlorodiammineplatinum(II), chlorozotocin, and 4000 rads of radiotherapy to the entire abdomen. She was treated with 70 mg/m^2 of *m*-AMSA and had clearing of malignant ascites and a decrease in a left pleural effusion. The ascites had previously required weekly paracentesis for patient comfort. This drug effect continued for 3 months (three doses of *m*-AMSA).

Clinical Pharmacology

One patient was studied at a dose level of 160 mg/m^2. Blood and urine concentrations of *m*-AMSA were followed using *m*-AMSA containing a ^{14}C-label in the 9-carbon of the acridine ring. Radioactive drug (37.7 microcuries) was added to the infusion bottle containing the nonlabeled drug and infused with the nonlabeled drug over a 1-hour period. The plasma levels of radioactivity were determined by digesting 0.1 ml of plasma with 1.0 ml of NCS tissue solubilizer (Amersham/Searle Corp, Arlington Heights, Ill), adding 18 ml of Aquasol, and counting in a Beckman LS-230 scintillation counter. Plasma samples were obtained at 5, 15, 30, and 45 minutes and 1, 1.5, 2, 4, 8, and 22 hours after the infusion.

Plasma radioactivity displayed an apparent biphasic decline with an early phase half-life of 11 minutes and a second phase half-life of 4.5 hours. Because total plasma radioactivity measured free *m*-AMSA, metabolized drug, and protein-bound drug, aliquots of plasma were shaken with ethyl acetate (1:10 vol/vol) and the free *m*-AMSA was extracted (11). Plasma levels of parent drug declined in a biphasic manner with an early phase half-life of 12 minutes and a second phase half-life of 2.5 hours. The difference between the second phase half-life of total plasma radioactivity (4.5 hours) and that of parent *m*-AMSA (2.5 hours) probably represents the presence of metabolized and protein-bound drug. Plasma samples taken 5 minutes after the end of the infusion measured 17 μM (6.7 μg/ml) in parent *m*-AMSA; samples obtained at 22 hours showed no detectable parent compound.

The urinary excretion of radioactivity was considerable, and 20% of the administered dose appeared in the urine within the first 8 hours following the start of the infusion. By the end of the third day after the infusion, urinary radioactivity accounted for 42% of the administered dose. Fecal excretion could not be determined because of narcotic-induced obstipation.

DISCUSSION

The results of this study indicate that *m*-AMSA has tolerable toxicity and can be given safely as a single dose every 28 days. The dose-limiting toxic effect was leukopenia. Thrombocytopenia was less common and was very minimal except in two patients who had had prior whole abdominal and pelvic radiotherapy. Phlebitis was the major nonmyelosuppressive toxic effect of *m*-AMSA. However, this side effect could be circumvented by diluting the dose of *m*-AMSA in 500 ml of 5% dextrose in water and infusing the drug over 1 hour.

m-AMSA is theoretically an attractive compound for clinical use. It has excellent antitumor activity in animal tumors, concentrates in the liver of experimental animals, binds to melanin granules in animal systems, has reproducible and reversible toxicity in man, and has shown some antitumor activity in this phase I trial. All of these features encourage initiation of phase II studies with *m*-AMSA to evaluate its activity against solid tumors and hematologic malignancies. The present phase I trial suggests that in patients previously treated with chemotherapy, the dose for phase II studies of *m*-AMSA should be 120 mg/m^2 as a single dose given on an every-4-week schedule. In patients treated with prior chemotherapy plus prior radiotherapy to the whole abdomen or pelvis, the starting dose should be 90 mg/m^2 every 4 weeks.

Future clinical work with this drug should involve an evaluation of the oral form of the drug. Oral *m*-AMSA has shown selective cytotoxic effects on L1210 cells in the livers of mice and is therefore attractive for testing in human tumors which metastasize to the liver (16). In addition, there are $>$ 120 analogs of *m*-AMSA which have already been described (4,17–21). If *m*-AMSA has antitumor activity in phase II studies, a number of its analogs may be worthy of further development.

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ADDENDUM

Since submission of this manuscript, a patient with nodular poorly differentiated lymphocytic lymphoma previously refractory to cyclophosphamide, vincristine, prednisone, adriamycin, and VP-16 has had a complete remission with *m*-AMSA at a dose of 120 mg/m^2.

Vindesine: A Phase II Study in the Treatment of Breast Carcinoma, Malignant Melanoma, and Other Tumors [1]

Ian E. Smith,* David W. Hedley, Trevor J. Powles, and Timothy J. McElwain [2,3]

SUMMARY

A phase II study of vindesine was carried out in 50 patients with histologically proven metastatic malignant disease, of whom 43 were evaluable for tumor response. Treatment was given at a starting dose of 3 mg/m^2 weekly by iv bolus with weekly 1-mg increases as toxicity allowed. The response rate in 21 evaluable patients with breast carcinoma was 29%, with two patients achieving a complete remission and four achieving a partial response (PR). Five other patients achieved a measurable response. Responses were seen most commonly in soft tissue, nodal, and pulmonary metastases. Two of 12 evaluable patients with malignant melanoma achieved a PR (16%) and a third achieved a measurable response. One of four evaluable patients with malignant teratoma of the testis achieved a PR and one achieved a measurable response. No responses were seen in three patients who had lymphoma or in one patient each who had rhabdomyosarcoma, osteosarcoma, nephroblastoma, and transitional cell carcinoma of the renal pelvis. Forty-seven patients were evaluable for toxicity. Leukopenia occurred in 21 patients (45%) and was dose-related; this was usually moderate and transient and was never associated with severe infection. Thrombocytopenia was rare, occurring in only two patients. Neurotoxicity occurred in 19 patients (40%) and most commonly presented with peripheral paresthesiae. Constipation occurred in eight patients (17%). Neurotoxicity was usually mild and nonprogressive but was eventually dose-limiting in four patients. Alopecia occurred in 23 patients (46%). It was concluded that vindesine was a clinically active agent with a spectrum of toxicity between that of vincristine and vinblastine.

[Cancer Treat Rep 62:1427–1433, 1978]

Vindesine is a new semi-synthetic vinca alkaloid derived from vinblastine, with a range of activity against experimental animal tumors greater than that of vinblastine and similar to that of vincristine, but with less neurotoxicity (1). Phase I studies in man have shown that a weekly dose of 2–4 mg/m^2 by iv bolus is well-tolerated (1,2) although other dose schedules are currently being evaluated (3,4). Preliminary reports have suggested clinical activity in the treatment of malignant melanoma (1,5), large cell carcinoma of the lung (1), acute lymphoblastic leukemia (4–7), acute myelomonocytic leukemia (5,6), lymphoma (6,7), nephroblastoma (4), and histiocytosis X (5). Only limited information is available on its activity against breast carcinoma.

We report here on a phase II study of vindesine in the treatment of 50 patients with metastatic malignant disease, including, in particular, breast carcinoma and malignant melanoma.

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[2] Division of Medicine, The Royal Marsden Hospital, Sutton, Surrey, England.

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**Reprint requests to:* Dr. Ian E. Smith, Division of Medicine, The Royal Marsden Hospital, Downs Rd, Sutton, Surrey SM2 5PT, England.

PATIENTS AND METHODS

Patient selection.—Fifty patients with histologically proven metastatic malignant disease were entered into this study between June 1977 and February 1978. Twenty-three patients had breast carcinoma, 14 had malignant melanoma, five had testicular teratoma, four had lymphoma, and one each had rhabdomyosarcoma, osteosarcoma, nephroblastoma, and transitional cell carcinoma of the renal pelvis. Thirty-seven patients had previously become resistant to conventional chemotherapy. Ten patients with minimal asymptomatic metastatic breast carcinoma involving soft tissues or pleura and three patients with melanoma were treated with vindesine as first-line chemotherapy. Clinical details of 21 breast carcinoma patients whose response to vindesine was evaluable are given in table 1. Informed consent was obtained from all patients.

Treatment schedule.—On the basis of phase I data (1,2), vindesine was given at an initial weekly dose of 3 mg/m^2 by iv bolus. The dose was then increased by 1 mg weekly as toxicity allowed. In five patients responding to treatment, the maintenance dose was changed after 2 months to 4 mg/m^2 every 2 weeks.

Investigations.—All patients were seen weekly and evaluated for response and toxicity. Investigations before and during treatment included weekly cbc counts, plasma urea and electrolytes, serum calcium, uric acid, bilirubin, alanine transferase, alkaline phosphatase, and total protein; chest roentgenograms and bone marrow examinations were done initially and repeated subsequently where indicated; skeletal roentgenograms, bipedal lymphography, bone scan, liver scan, liver ultrasound, and estimations of serum β-subunit of human chorionic gonadotrophin and α-fetoprotein were also done when appropriate.

Criteria for response.—Patients who received treatment for at least 4 weeks, or who showed clear evidence of disease progression after at least 2 weeks of treatment, were considered evaluable for response. Three categories of response were defined. *Complete remission* (CR) was defined as the disappearance of all measurable disease for at least 1 month, assessed both clinically and by appropriate investigations; *partial response* (PR) was defined as a > 50% reduction in the sum of the product of the diameters of measurable lesions for at least 1 month, with no new lesions appearing; *measurable response* was defined as a < 50% reduction in the products of the diameters of each lesion or a > 50% reduction in some lesions but no change in others, with no new lesions appearing. *Response in bone* was defined on the basis of radiologic evidence of recalcification or the disappearance of "hot spots" on an isotopic bone scan; it was considered impractical to subdivide this into CR or PR. For patients with breast carcinoma, the criteria for CR and PR were based on the International Union Against Cancer system for evaluating response to treatment in this disease (7).

RESULTS

Of the 50 patients entered into the study, 43 were evaluable for response and 47 for toxicity; of the seven who had an inadequate therapeutic trial, three died within 3 weeks of starting treatment and four had treatment discontinued after fewer than four courses because of toxicity (*see* below).

Response in breast carcinoma (table 2).—Two of the 21 evaluable patients with metastatic disease achieved a CR and four achieved a PR, giving an overall response rate of 29%. Five other patients achieved a measurable response so that, in all, 11 patients (52%) improved. Six of these 11 patients had received no previous chemotherapy. Response by site in patients with breast carcinoma is shown in table 1.

Response in melanoma (table 2).—Two of the 12 evaluable patients (16%) with melanoma achieved a PR; one had liver and the other had pulmonary involvement. Both patients had previously failed to respond to DTIC. A third patient had a measurable response with improvement in liver function and in hemiparesis secondary to cerebral metastases.

Response in other tumors (table 2).—One patient with malignant teratoma involving the lungs and lymph nodes achieved a PR and a second achieved a measurable response of the lymph nodes.

Response duration and previous vinca alkaloid therapy.—Details of these parameters for all patients achieving a CR or PR to vindesine are given in table 3. Only one responding patient, with metastatic teratoma, had previously become resistant to vinblastine, and none was resistant to vincristine.

Toxicity

Forty-seven patients were evaluable for toxicity (table 4). Leukopenia (< 3000 wbcs/mm^3) occurred in 21 patients, more commonly when the dose of vindesine was increased to 4 mg/m^2. This occurred within 7 days of treatment, and was usually moderate and transient; it was never associated with infection or septicemia. Thrombocytopenia was very rare, occurring in only two patients, both of whom had marrow infiltration and had received extensive previous chemotherapy; bleeding and purpura did not occur. Conversely, 13 patients had a significant

TABLE 1.—**Age, endocrine status, site(s) of disease, and details of previous endocrine therapy and chemotherapy in 21 breast carcinoma patients with evaluable response to vindesine**

Patient No.	Age in yrs	Endocrine status	Previous endocrine therapy (response)	Previous chemotherapy* (response)	Site(s) of disease	Vindesine response
1	52	Postmenopausal	Oophorectomy, adjuvant	—	Lung	CR
2	51	Postmenopausal	Estrogen (PR)	—	Lung	CR
3	56	Postmenopausal	Anti-estrogen (none)	—	Soft tissue	PR
4	57	Postmenopausal	Oophorectomy (none)	CMF (CR) VAP (CR)	Soft tissue Nodes Lung	PR PR CR
5	67	Postmenopausal	Estrogen (none) Progestogen (none)	—	Soft tissue Node	PR PR
6	45	Postmenopausal	Oophorectomy (none)	—	Soft tissue Bone	PR PR
7	62	Postmenopausal	Estrogen (PR)	VAP (CR)	Soft tissue Node	Measurable PR
8	54	Postmenopausal	Estrogen (none)	CMFP (PR) VAP (PR)	Soft tissue Node Liver Bone Marrow	None PR None None None
9	34	Premenopausal	—	CMF (none)	Soft tissue Lung	PR None
10	69	Postmenopausal	—	CMF (PR)	Soft tissue Lung	Measurable PR
11	58	Postmenopausal	—	—	Soft tissue Node	Measurable Measurable
12	53	Postmenopausal	—	CMFP (none)	Soft tissue Bone	None None
13	70	Postmenopausal	Estrogen (none)	—	Lung	None
14	51	Perimenopausal	—	—	Soft tissue Node	None None
15	50	Postmenopausal	Estrogen (PR)	VAP (none) CMF (none)	Soft tissue Node Lung	None None None
16	57	Postmenopausal	—	VAP (CR)	Lung	None
17	52	Postmenopausal	Adrenalectomy (PR)	—	Soft tissue Bone Marrow	None None None
18	60	Postmenopausal	—	CMAFV (CR)	Lung	None
19	54	Postmenopausal	Anti-estrogen (PR)	CMFP (none)	Lung Bone Marrow	None None None

TABLE 1.—Age, endocrine status, site(s) of disease, and details of previous endocrine therapy and chemotherapy in 21 breast carcinoma patients with evaluable response to vindesine—Continued

Patient No.	Age in yrs	Endocrine status	Previous endocrine therapy (response)	Previous chemotherapy* (response)	Site(s) of disease	Vindesine response
20	49	Perimenopausal	—	CMF, adjuvant	Soft tissue	None
					Liver	None
21	50	Postmenopausal	—	—	Lung	None

*V = vincristine; A = adriamycin; P = prednisone; C = cyclophosphamide; M = methotrexate; and F = 5-fluorouracil.

TABLE 2.—Phase II study of vindesine: response by disease type

Tumor	Total No. of patients	No. of patients				
		Evaluable	Response			
			CR	PR	Measurable	None
Breast carcinoma	23	21	2 (10%)	4 (19%)	5	10
Melanoma	14	12	0	2 (16%)	1	9
Teratoma	5	4	0	1 (25%)	1	2
Lymphoma	4	3	0	0	0	2
Transitional cell carcinoma	1	1	0	0	0	1
Rhabdomyosarcoma	1	0	—	—	—	—
Osteosarcoma	1	1	0	0	0	1
Nephroblastoma	1	1	0	0	0	1

TABLE 3.—Response to previous vinca alkaloid therapy, total vindesine dose, and response duration in patients achieving CR or PR to vindesine

Patient No.	Tumor	Previous vincristine-vinblastine therapy	Vindesine response	Total dose (mg/m²)	Response duration (mos)
1	Breast carcinoma	—	CR	34	3½*
2	Breast carcinoma	—	CR	15	2*
3	Breast carcinoma	—	PR	64	5*
4	Breast carcinoma	Vincristine (response)	PR	24	3
5	Breast carcinoma	—	PR	36	3½*
6	Breast carcinoma	—	PR	70	8
22	Melanoma (lung)	—	PR	35	6
23	Melanoma (liver)	—	PR	25	4
24	Teratoma (lung)	Vinblastine (response, then relapse)	PR	45	2

*Patients with continuing response and changed to combination chemotherapy.

increase in platelet count (by at least 100,000/mm^3) during the first three courses of chemotherapy although frank thrombocytosis (> 350,000 cells/mm^3) was not seen.

Peripheral paresthesiae were common, occurring in 19 patients (40%) and resembling clinically the neurotoxicity associated with vincristine. Symptoms developed after three or four courses of treatment but were usually mild and only slowly progressive, particularly in patients in whom maintenance treatment was given at 2-week intervals in a larger dose (4 mg/m^2). Neurotoxicity was severe enough to stop treatment in four patients: one developed an ileus with associated abdominal and jaw pain after two courses and 6 mg/m^2 of treatment; one developed severe atypical generalized painful paresthesiae after a single 3-mg/m^2 course of treatment; two developed progressive numbness in the fingertips and impairment of fine hand movements after five weekly courses (15 mg/m^2, total) and eight weekly courses (24 mg/m^2, total), respectively (interestingly, both of these patients achieved a CR). Constipation was not a major problem and only occurred to a significant extent in eight patients (17%). Symptoms

TABLE 4.—Vindesine toxicity in 47 patients

Toxic effect	No. of patients	(%)
Leukopenia (cells/mm^3)		
2000–3000	15	(45)
1000–1999	6	
< 1000	0	
Thrombocytopenia (platelets/mm^3)*		
< 100,000	2	(4)
Neurotoxic effects		
Paresthesiae	19	(40)
Constipation	8	(17)
Ileus	1	(2)
Loss of taste	1	(2)
Jaw pain	1	(2)
Miscellaneous		
Alopecia	23	(49)
Nausea	10	(21)
Vomiting	3	(6)
Fatigue	10	(21)
Rash	3	(6)
Myalgia	1	(2)

*13 patients (28%) had an increase in platelet count > 100,000/mm^3 during the first 3 courses of treatment.

associated with neurotoxicity usually began to improve within 2 weeks of discontinuing therapy and even in severe cases they were resolved within 2 months.

Alopecia was common, occurring in 23 patients overall and to some extent in all but three patients who completed more than six courses of treatment. This alopecia was never complete, but nevertheless necessitated the use of a wig in 14 patients. Nausea was uncommon, and was usually mild and transient; it was associated with brief episodes of vomiting in only three patients. Ten patients (21%) complained of fatigue and malaise for 3 or 4 days after treatment but only one complained of myalgia. These symptoms were relieved in three patients with prednisolone at a daily dose of 20 mg for 3 days after treatment. Three patients developed an itchy erythematous maculopapular rash over the trunk after the second course of treatment; in two patients this ended spontaneously without interruption of therapy and in the third patient treatment was discontinued because of the development of an ileus (*see* above).

Epileptiform attacks.—Four patients with cerebral deposits from melanoma had either grand mal or Jacksonian seizures within 1 week after receiving vindesine. Two of these patients had seizures at 6 and 8 hours, respectively, after vindesine on two separate occasions but tolerated other courses uneventfully.

DISCUSSION

The 29% CR and PR rate for vindesine in the treatment of breast cancer was at least as good as that previously described for vincristine or vinblastine (8), and the observation that > 50% of patients achieved at least some degree of tumor regression was encouraging. It must be noted however that five of the six responding patients had received no previous chemotherapy. Further phase II data are now required for vindesine in breast carcinoma.

The response rate in melanoma was no better than that for other single-agent chemotherapy, albeit in a small number of patients. It is of some interest that two responses were seen in patients resistant to DTIC, contradicting previous experience (9,10), and the relative lack of toxicity of vindesine compared with DTIC justifies further study in melanoma. Little activity was seen against the other tumors studied, although all patients in this group had advanced disease resistant to conventional chemotherapy. The observation that one patient with metastatic teratoma resistant to another vinca alkaloid (vinblastine) nevertheless responded to vindesine suggests that the drug may not always show cross-resistance with the other vinca alkaloids.

The toxicity of vindesine was similar to that of the other vinca alkaloids. The drug was well-tolerated, with nausea or vomiting uncommon and transient. Leukopenia was less marked than that usually associated with vinblastine and was never associated with infection. The peripheral platelet count was more likely to rise than to fall with treatment, resembling experience with the other vinca alkaloids (11). Neurotoxicity was common but less severe than might have been anticipated with vincristine: paresthesiae were moderate, only slowly progressive, and readily reversible in most patients receiving weekly treatment often for several months; constipation was uncommon. Furthermore, our limited experience with five patients receiving a higher maintenance dose treatment at 2-week intervals suggested that neurotoxicity might be further reduced with this type of schedule, which is commonly used in combination chemotherapy.

In conclusion, vindesine is a clinically active agent, particularly in the treatment of breast carcinoma, with a spectrum of toxicity which lies between that of vincristine and vinblastine. The possible advantages of its substitution for either of the latter agents in combination regimens remain to be explored.

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Comparison of the Therapeutic Effects of Adriamycin Alone Versus Adriamycin Plus Vincristine Versus Adriamycin Plus Cyclophosphamide in the Treatment of Advanced Carcinoma of the Cervix[1,2]

H. J. Wallace, Jr, M. M. Hreshchyshyn, G. D. Wilbanks, R. C. Boronow, W. C. Fowler, Jr, and J. A. Blessing[3,4,*]

SUMMARY

Three hundred and twenty-six patients with advanced recurrent or metastatic squamous cell carcinoma of the cervix were entered in a randomized controlled study comparing adriamycin alone (60 mg/m^2 iv), adriamycin (60 mg/m^2 iv) plus vincristine (1.5 mg/m^2 iv), and adriamycin (50 mg/m^2 iv) plus cyclophosphamide (500 mg/m^2 iv). Each course was repeated at 3–4-week intervals until tumor progression was noted or a maximum cumulative dose of 550 mg/m^2 of adriamycin was reached. One hundred and seventy-nine patients were evaluated. Twenty-five percent demonstrated severe to life-threatening leukopenia while only 4% demonstrated the same degree of thrombocytopenia. Cardiac toxicity occurred in only six instances and did not result in death. Four complete responses and 12 partial responses were observed in patients with pelvic recurrence; seven complete responses and five partial responses were observed in patients with extrapelvic metastases. There were no significant differences in response rates between the pelvic and extrapelvic tumor in the three regimens. The median progression-free interval ranged from 3.3 to 3.9 months and the median survival from 5.9 to 7.3 months. None of these differences were significant and it is apparent that adriamycin alone or in combination with vincristine or cyclophosphamide at maximum tolerated doses has little activity in the treatment of advanced, recurrent, and metastatic squamous cell carcinoma of the cervix.

[Cancer Treat Rep 62:1435–1441, 1978]

Recurrent or metastatic squamous cell carcinoma of the uterine cervix is seen most commonly following curative attempts utilizing radical surgery, high-dose pelvic irradiation therapy, or a combination of both. Chemotherapeutic success in dealing with cervical cancer has been disappointing, in part due to the extensive prior treatment, and in part due to the relative resistance of this histologic type to the available chemotherapeutic agents. There has been some experience with almost all standard chemotherapeutic agents in the treatment of advanced squamous cell carcinoma of the cervix, with relatively few drugs showing significant and consistent activity.

Adriamycin is one of the most highly active anticancer agents in a broad spectrum of solid tumors when used either as a single agent or in combination with other drugs. The reported response rates in squamous cell carcinoma of the cervix using adriamycin alone have ranged widely from < 10% (1) and 20% (2) to up to 40% (3). Blum

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[3] This study was conducted by the Gynecologic Oncology Group. The following institutions participated in the study: Roswell Park Memorial Institute, Buffalo, NY (*H. J. Wallace, Jr*, and *J. A. Blessing* [group statistician]); State University of New York at Buffalo (*M. M. Hreshchyshyn*); Rush Medical College, Rush-Presbyterian-St. Luke's Medical Center, Chicago, Ill (*G. D. Wilbanks*); University of Mississippi Medical School, Jackson (*R. C. Boronow*); and University of North Carolina School of Medicine, Chapel Hill (*W. C. Fowler, Jr*).

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[*] *Reprint requests to:* Gynecologic Oncology Group Headquarters, PO Box 60, Philadelphia, Pa 19150.

and Carter (4) reviewed the literature and found a response rate of 32% (nine of 28 patients), which ranked adriamycin as potentially one of the most effective drugs for this disease. This indication of significant activity of adriamycin in the small numbers of reported patients warranted confirmation of its activity as a single agent in a larger, randomized, controlled trial. The safety, efficacy, and convenience of an intermittent single, high-dose adriamycin schedule had been demonstrated (5). Adriamycin in full dose, however, causes significant myelosuppression with the added risk of causing severe cardiomyopathy in cumulative doses > 550 mg/m^2 of body surface area.

Several other drugs have shown evidence of activity in cervical cancer, one of which is vincristine. In a phase I–II trial, Hreshchyshyn reported 12 objective responses in 45 patients treated at dose levels between 50 and 75 μg/kg/week (6). These responses were achieved at the cost of considerable neurotoxicity but the lack of significant bone marrow toxicity made vincristine a leading candidate for combination with adriamycin as the second arm of a controlled trial.

Cyclophosphamide is another agent that has shown some evidence of antitumor activity in this disease when given in large doses. Solidoro et al (7) reported a 10% response rate in 42 patients at a dose level of 3–4.5 mg/kg/day iv until moderate leukopenia occurred. The patients were then maintained with daily oral cyclophosphamide until relapse. Smith et al (8) noted a somewhat higher response rate of 20% in 76 patients treated at a dose level of 8 mg/kg/day for 5 days and repeated at 4–6-week intervals. The Gynecologic Oncology Group felt that this minimal antitumor activity need not be confirmed by using cyclophosphamide as a single agent, since any improvement in response rate would require more intensive dose schedules with a hazard of more serious myelosuppression and hemorrhagic cystitis in patients who had previously had heavy pelvic irradiation. Trials using the combination of cyclophosphamide plus adriamycin were underway and indicated a tolerable dose level and evidence of significant anticancer activity (9,10). It was therefore elected to include the combination of adriamycin and cyclophosphamide as the third arm of the study.

The Gynecologic Oncology Group designed protocol No. 23 as a randomized controlled study to compare the effects of adriamycin alone and in combination with either vincristine or cyclophosphamide in the treatment of advanced squamous cell carcinoma of the uterine cervix. The group hoped to demonstrate a greater response rate and survival by combining drugs with different mechanisms of action, spectra of antitumor activity, and toxicities.

MATERIALS AND METHODS

Between September 10, 1974, and June 4, 1976, 326 patients with recurrent or metastatic squamous cell carcinoma of the uterine cervix, who were no longer candidates for either radiotherapy or surgery, were entered into protocol No. 23. Advanced, recurrent cervical cancer is frequently difficult to measure accurately because of the fibrosis following pelvic irradiation and surgery. Therefore, measurable disease was not a requirement for protocol entry. Consequently, progression-free interval and survival were the main criteria for evaluation. Every effort was made to measure disease where possible prior to initiation of chemotherapy and before each subsequent course by pelvic examination and other clinical examinations or diagnostic roentgenogram. Patients were required to have an estimated survival of at least 2 months with a wbc count of at least 4000/mm^3 and a platelet count of at least 150,000/mm^3. A serum creatinine level > 1.5 mg/100 ml or a BUN level > 25 mg/100 ml made the patients ineligible for the study, as did hepatic failure characterized by a bilirubin level > 2.0 mg/100 ml. Patients with second primary cancer or patients who had received adriamycin, vincristine, or cyclophosphamide previously were also ineligible. No chemotherapy or radiation therapy was given during the preceding 2 weeks and all therapeutic and toxic side effects from previous treatment had subsided.

The patients were randomly assigned to one of three treatment regimens in balanced groups of six by the closed-envelope technique. Single, high-dose adriamycin alone or in combination with vincristine or cyclophosphamide was given according to the schema shown in table 1. Courses were repeated at 3–4-week intervals with dose modifications based on nadir wbc and platelet counts. If the nadir wbc count was ≥ 3000/mm^3 and the nadir platelet count

TABLE 1.—Regimens tested

Regimen	Drugs	Dosage
I	Adriamycin	60 mg/m^2 iv q3wks
II	Adriamycin +	60 mg/m^2 iv q3wks
	Vincristine	1.5 mg/m^2 iv q3wks (maximum dose, 2.5 mg/m^2)
III	Adriamycin +	50 mg/m^2 iv q3wks
	Cyclophosphamide	500 mg/m^2 iv q3wks

TABLE 2.—Patient data

Regimen	No. of patients— Entered	Ineligible	Inevaluable	No pathology review	Not excluded from evaluation	Unable to tolerate therapy	Total No. evaluable for survival and progression-free interval
I	118	20	30	5	63	2	61
II	109	20	23	3	63	2	61
III	99	25	16	5	53	1	52
Totals	326	65	69	13	179	5	174

was ≥ 100,000/mm^3, the dose of adriamycin was escalated in regimens I and II to 75 mg/m^2 and then to 90 mg/m^2 if tolerated. In regimen III, the adriamycin dose escalation was to 60 mg/m^2 and then to 70 mg/m^2 if the same conditions existed. Vincristine and cyclophosphamide doses were not escalated. The dose remained constant if the nadir wbc count was between 2000/mm^3 and 3000/mm^3 and the nadir platelet count was between 50,000/mm^3 and 100,000/mm^3. Below those levels, the adriamycin and cyclophosphamide doses were reduced by 50%. Vincristine doses were decreased by 50% if deep tendon reflexes disappeared accompanied by diminution of muscle strength by 50%, and were temporarily omitted if there was progression of neurotoxicity. Dose de-escalation was carried out if there was severe gastrointestinal or mucous membrane toxicity. Serial ECGs were carried out and adriamycin was discontinued if there was any evidence of cardiac toxicity characterized by evidence of congestive cardiac failure or persistent electrocardiographic changes. Adriamycin was discontinued at a cumulative dose level of 550 mg/m^2.

Vincristine dose was decreased by 75% in patients with obstructive jaundice and by 50% in patients with hepatocellular jaundice, and the adriamycin dose was decreased by 25% in these instances.

Adequate fluid intake and water diuresis were encouraged on the day of cyclophosphamide injection to decrease the risk of hemorrhagic cystitis and/or bladder fibrosis in view of the previous heavy irradiation and pelvic surgery. If evidence of hemorrhagic cystitis occurred, subsequent doses of cyclophosphamide were delayed until all evidence subsided, and then patients were restarted at a 50% decrease in dose.

Patients were scheduled to receive a minimum of two courses of chemotherapy at which time chemotherapy was continued if the patients demonstrated static disease or evidence of tumor regression. All chemotherapy was discontinued when tumor progression was observed or when a maximum cumulative dose of 550 mg/m^2 of adriamycin was reached.

Tumors were measured when possible and therapeutic response was determined by the sum of the products of the longest diameter and the perpendicular diameter. Complete regression was defined as the complete disappearance of all measurable tumor and relevant symptoms. Partial regression was a > 50% reduction in the area of at least one tumor with no progression or new tumors noted elsewhere. Improvement was defined as between 25% and 50% reduction in the area of at least one tumor with no progression or new tumors noted elsewhere. Static disease was a < 25% change in all tumors, and progression was defined as a > 25% increase in the area of one measurable tumor or the appearance of a new lesion. Any change in tumor measurement had to persist for at least 3 weeks to be of significance.

The progression-free interval was defined as the period from the start of chemotherapy to objective evidence of tumor progression. Survival was defined as the period from the start of chemotherapy to death.

Wbc count toxicity was defined as follows: moderate (2000–2999/mm^3), severe (1000–1999/mm^3), and life-threatening (< 1000/mm^3). Platelet count toxicity was defined as follows: moderate (50,000–99,999/mm^3), severe (25,000–49,999/mm^3), and life-threatening (< 25,000/mm^3).

RESULTS

The completed Gynecologic Oncology Group records for 326 patients entered were reviewed. The disposition of these cases is shown in table 2. One hundred and seventy-four patients were evaluable for survival and progression-free interval. Of these, 136 had measurable disease and were considered evaluable for response. Sixty-one patients each received regimens I and II and 52 patients received regimen III. A total of 65 patients were excluded as ineligible, more than half of these because of the diagnosis of adenosquamous carcinoma rather than squamous cell carcinoma after review by the Pa-

TABLE 3.—Wbc count toxicity according to treatment

	No. of patients with toxicity grade—					
Regimen	None	Mild	Moderate	Severe	Life-threatening	Total
I	24	10	10	11	4	59
II	24	7	13	7	8	59
III	19	7	12	8	4	50
Totals	67	24	35	26	16	168

TABLE 4.—Platelet count toxicity according to treatment

	No. of patients with toxicity grade—					
Regimen	None	Mild	Moderate	Severe	Life-threatening	Total
I	46	4	3	3	1	57
II	51	5	0	1	1	58
III	45	3	0	1	0	49
Totals	142	12	3	5	2	164

thology Committee. The remainder were excluded because of a second primary, wrong primary, elevated BUN or creatinine level, poor risk at entry, or no evidence of progression following previous therapy. Sixty-nine patients were inevaluable because inadequate data were collected, therapy was never initiated, there was insufficient followup after the first course, or because of a number of other less frequent occurrences such as improper randomization and clerical errors.

Toxicity

The records of 179 patients were reviewed for toxicity. One hundred and sixty-eight patients were evaluable for wbc count toxicity and 164 were evaluable for platelet count toxicity. The myelosuppressive toxicity was based both on the wbc and platelet counts just prior to the subsequent course of chemotherapy and on nadir counts, when available. Wbc count toxicity is shown in table 3 and platelet count toxicity in table 4. It is significant that 46% of the patients demonstrated moderate, severe, or life-threatening leukopenia while only 6% demonstrated moderate, severe, or life-threatening thrombocytopenia. The leukopenia was comparable in all three regimens while adriamycin alone (regimen I) had a higher incidence of thrombocytopenia. The numbers are small but the data suggest that the combination of adriamycin with either vincristine or cyclophosphamide had a platelet-sparing effect.

Possible cardiac toxicity was recognized in only six patients (3.8%) and was characterized by evidence of congestive cardiac failure (two patients) or decreased voltage and other electrocardiographic abnormalities (four patients). These cardiac changes occurred at cumulative adriamycin doses of between 120 and 285 mg/m^2. Cardiac failure did not contribute to death in any of the patients, indicating the value of limiting the cumulative dose of adriamycin to 550 mg/m^2. There was no evidence that cyclophosphamide added to the cardiac toxicity, since four of the six patients were receiving the adriamycin-vincristine regimen and one each was receiving the other two regimens.

Thirty-eight percent of the patients were treated for ≤ 3 months. The treatment was discontinued in these patients after one to three courses because of progression, death, or other causes. Thirty percent of the patients received four to six courses and 32% received seven or more courses, up to a maximum of 12 courses. There were no differences between the three regimens in the number of courses received.

Slightly more than half of the patients received between 91% and 100% of the scheduled dose of drug(s) required in the protocol. Twenty-six percent of the patients had the adriamycin dose escalated because of minimal toxicity during the preceding course. Nineteen percent of the patients received $\leq 90\%$ of the scheduled drug(s) because of dose de-escalation required by severe or greater toxicity.

Response

There were 136 patients evaluable for tumor response. Eighty-six of these patients had only

TABLE 5.—Response according to treatment: pelvic disease

Regimen	No. of patients with— Complete response	Partial response	Improvement	No change	Progression	Total
I	2	4	0	16	16	38
II	1	5	1	14	17	38
III	1	3	3	11	10	28
Totals	4	12	4	41	43	104

TABLE 6.—Response according to treatment: extrapelvic disease

Regimen	No. of patients with— Complete response	Partial response	Improvement	No change	Progression	Total
I	5	1	0	9	8	23
II	0	3	0	8	5	16
III	2	1	0	4	4	11
Totals	7	5	0	21	17	50

pelvic recurrence and extension, 32 had only metastatic disease outside the pelvis, and 18 had recurrent disease in the pelvis and metastatic disease elsewhere. Because of the possible interference with a favorable response in disease recurring in the pelvis following extensive surgery and radiation therapy, pelvic and extrapelvic disease were evaluated separately for response. Table 5 shows the number of patients with pelvic recurrence and indicates a disappointing overall complete and partial response rate of 15.4%. No differences in response rate were noted among the three treatment regimens. Extrapelvic metastases (table 6) demonstrated a somewhat greater overall complete and partial response rate of 24%. Regimens I and III demonstrated more complete responses than partial responses, but because of the small numbers these differences are not significant.

Progression-Free Interval

The median progression-free interval for regimens I, II, and III was 3.3, 3.4, and 3.9 months respectively. These differences are not significant and are graphically shown in figure 1. The slopes of these curves do not differ when they are calculated to compare the 148 patients who had received prior radiation therapy versus the 14 patients who had received no prior radiation therapy.

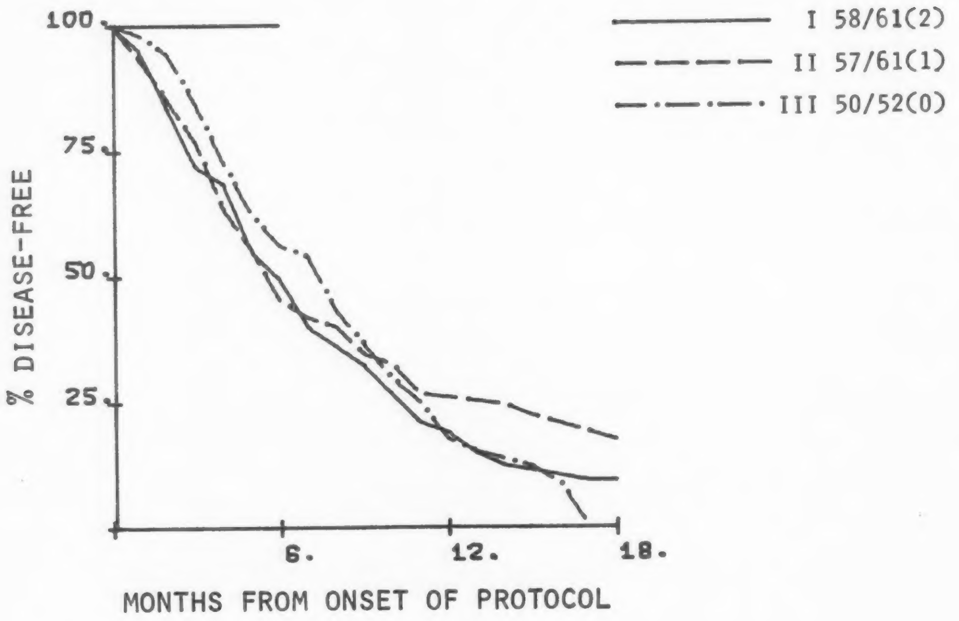
Survival

Median survival for the three regimens was 5.9, 5.5, and 7.3 months respectively. Again, these differences are not significant as is graphically shown in figure 2. Comparison of those patients receiving prior radiation therapy with the small number of patients who received no prior radiation therapy also shows no prior treatment effect of significance.

DISCUSSION

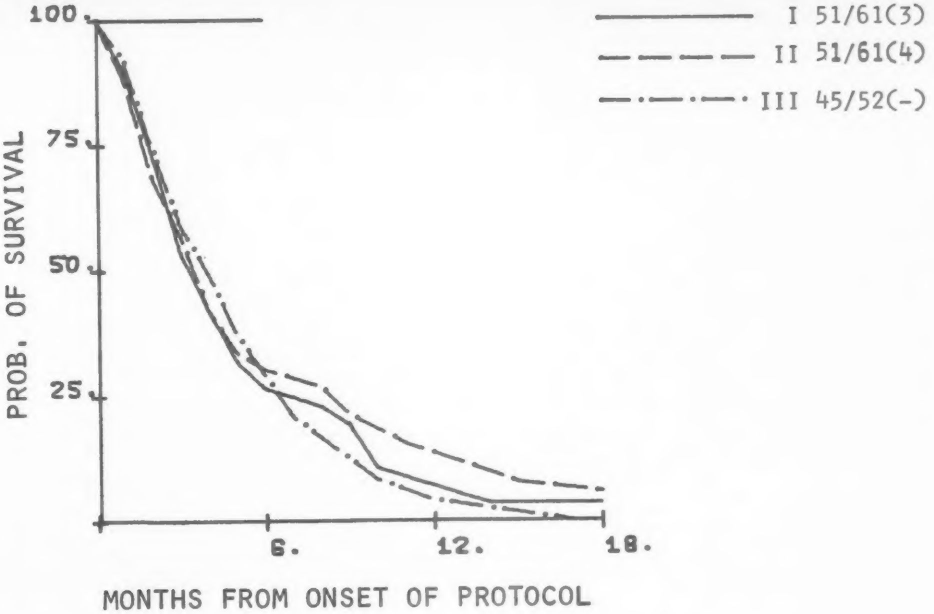
The Gynecologic Oncology Group has completed a randomized comparison of adriamycin alone versus adriamycin plus vincristine versus adriamycin plus cyclophosphamide in the treatment of advanced recurrent and metastatic squamous cell carcinoma of the uterine cervix. The data for 174 evaluable patients indicate that the three treatment regimens are well-tolerated in spite of the fact that most of the patients had had previous radical surgery and 90% had received extensive pelvic irradiation. The major dose-limiting toxicity was leukopenia, with one quarter of the patients demonstrating severe or life-threatening toxicity. Thrombocytopenia was surprisingly infrequent as were cardiac toxicity and hemorrhagic cystitis.

The median progression-free interval of about 3.5 months and the median survival of about 6 months were disappointingly brief and showed no differences among the three treatment regimens. It is apparent that adriamycin as a single agent or in combination with either vincristine or cyclophosphamide at doses causing significant myelosuppression is only somewhat effective for advanced squamous cell carcinoma of the cervix. It seems unlikely that adriamycin, vincristine, or cyclophosphamide



Fractions show recurrences/entries.

FIGURE 1.—Progression-free interval according to therapy.



Fractions show deaths/entries.

FIGURE 2.—Survival probability according to therapy.

would add significantly to the therapeutic potential of other drug combinations for this disease. The accession of 326 patients with advanced, recurrent, or metastatic squamous cell carcinoma of the cervix from 29 institutions in < 2 years is an indication of the need to continue the search for more effective chemotherapeutic treatments for advanced disease and for adjuvant use in patients with a high risk of recurrence.

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Correlation of Tubulin-Binding and Antitumor Activities of Podophyllotoxin Analogs[1,2]

Joanne K. Kelleher[3,4,*]

SUMMARY

The tubulin-binding affinities of podophyllotoxin and 12 related compounds have been compared with the effectiveness of these drugs in three assays of antitumor activity: (1) The mastocytoma assay of inhibited tumor cell growth in vitro; (2) the 37 sarcoma assay of tumor remission in vivo; and (3) the *Stentor* regeneration inhibition assay which detects a requirement for microtubule polymerization. The results indicate that there is a positive Spearman rank correlation coefficient between the inhibition constant for the binding of colchicine to tubulin in the presence of these compounds and the effectiveness of the compounds in the three assays of tumor growth inhibition. It is suggested that tubulin-binding assays may be a useful step in the screening of podophyllotoxin-like compounds suspected of disrupting mitosis by binding to microtubule protein.

[Cancer Treat Rep 62:1443–1447, 1978]

Microtubules form the mitotic spindle necessary for proper metaphase alignment and subsequent separation of chromosomes. Thus, the polymerization of microtubules is an essential requirement for cell division in eukaryotes. Certain compounds, including colchicine, podophyllotoxin, and vinblastine, bind to microtubule protein, tubulin, and inhibit mitosis (1). These drugs produce a characteristic arrested state of cell division termed "C-metaphase" (2). Colchicine and podophyllotoxin bind tubulin competitively (3,4) and probably occupy overlapping binding sites (5). The tubulin-binding characteristics of analogs of colchicine (6) and podophyllotoxin (7) have been investigated. These studies indicate that the affinity for tubulin varies with the drug structure and that analogs containing bulky or hydrophilic substitutions do not readily bind vertebrate tubulin.

It is important to note that tubulin-binding activity is not the only pharmacologic activity of this class of drugs. Colchicine and podophyllotoxin inhibit nucleoside transport in mammalian cells (8,9). One podophyllotoxin analog, VP-16-213, induces single-strand breaks in HeLa cell DNA (10). Therefore, not all biologic effects of these drugs are directly related to microtubules.

Because of their dramatic effect on mitosis, drugs which bind tubulin have been cited as potentially useful antineoplastic agents (11,12). If the mechanisms of action of a class of compounds involve binding microtubule protein, it is predicted that there will be a positive correlation between the affinity of the compounds for tubulin and their effectiveness as inhibitors of tumor cell growth. In this paper, the prediction is tested for colchicine and 12 podophyllotoxin-like compounds. Previous studies have demonstrated that there is a significant correlation between these two parameters for colchicine analogs (6).

METHODS AND MATERIALS

The drugs examined for this paper (figs 1 and 2) have been assayed using several techniques aimed at elucidating their mode of action in vivo. Tubulin-

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[3] Department of Biology, Boston University, Mass.

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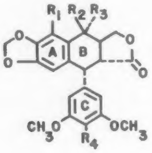
[*] *Reprint requests to:* Joanne K. Kelleher, PhD, Department of Developmental Biology, Boston Biomedical Research Institute, 20 Staniford St, Boston, Mass 02114.

binding constants have been measured with mouse-brain tubulin in vitro (7). In the standard assay, mouse-brain tubulin is incubated with ^{3}H-colchicine and a podophyllotoxin analog until equilibrium is reached. Colchicine-bound and free tubulin are then separated by a DEAE filter paper technique (13). From the degree of inhibition of colchicine binding in the presence of each podophyllotoxin analog, the dissociation constant for the drug-tubulin interaction is calculated. Since colchicine and podophyllotoxin bind competitively to tubulin, the inhibition constant for podophyllotoxin binding is determined experimentally from double reciprocal plots of colchicine binding in the presence of a competitive inhibitor according to conventional procedures for competitive inhibitors of enzyme activity (7). The K_i so determined is assumed to be equivalent to the K_d.

Cellular antitumor activity has been measured in tissue culture utilizing the mastocytoma assay of Stähelin (14). With this assay, the 50% effective dose, ED50, is estimated from the inhibition of in vitro cell growth in the presence of varying amounts of each drug. Antitumor activity in vivo has been evaluated by Leiter et al (15) and reported by Hartwell and Schrecker (16). This assay measures the minimum effective dose required to produce tumor remission after one injection of drug in the body cavity of tumor-bearing mice. In addition to these three assays utilizing mice and mouse tissue, the inhibition of a specific microtubule-dependent process in the protozoan cell, *Stentor coeruleus*, has been examined with many of these compounds. The *Stentor* assay determines the relative effectiveness of drugs as inhibitors of oral regeneration, a process requiring the formation of thousands of new cilia (17,18).



Analogue	R1	R2	R3	R4
podophyllotoxin	H	H	OH	OCH_3
β-peltatin	OH	H	H	OCH_3
β-peltatin-A-methyl ether	OCH_3	H	H	OCH_3
desoxypodophyllotoxin	H	H	H	OCH_3
epipodophyllotoxin	H	OH	H	OCH_3
4'demethylpodophyllotoxin	H	H	OH	OH
podophyllotoxin-β-D-glucopyranoside	H	H	$C_6H_{11}O_6$	OCH_3
β-peltatin-β-D-glucopyranoside	$C_6H_{11}O_6$	H	H	OCH_3
podophyllotoxin-4,6-O-benzylidene-β-D-glucoside	H	H	$\phi C_7H_{10}O_6$	OCH_3

FIGURE 1.—Structure of podophyllotoxin and 8 analogs.

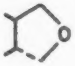
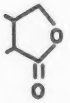
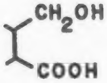
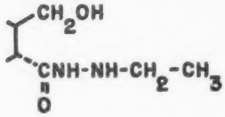
Analogue:	Lactone ring becomes:
anhydropodophyllol	
picropodophyllin	
picropodophyllic acid	
podophyllic acid-2-ethylhydrazide	

FIGURE 2.—Structure of podophyllotoxin analogs with alterations in the lactone ring.

RESULTS AND DISCUSSION

The results of these four assay techniques for podophyllotoxin analogs and colchicine are compared in table 1. It is apparent that compounds with lower dissociation constants (K_d), that is, those which bind tightest to tubulin, are generally the more effective compounds in the other assays. Spearman rank correlation coefficients were calculated and the level of significance was determined for various pairs of assays. Significant positive correlations were obtained when the dissociation constants were paired with either (*a*) the in vitro tumor assay ($P \leq 0.01$), (*b*) the in vivo tumor assay ($P \leq 0.02$), or (*c*) the minimum dose required to delay *Stentor* regeneration ($P \leq 0.02$). A significant rank correlation coefficient was also obtained by

TABLE 1.—Relative effectiveness of podophyllotoxin analogs in microtubule polymerization processes[*]

Drug	K_d[†] (μM)	ED50: inhibition of mastocytoma growth in vitro[‡] (μM)	Minimum dose damaging mouse sarcoma in vivo[§] (μmol/g of body weight)	Minimum dose delaying *Stentor* regeneration[¶] (μM)
β-Peltatin	0.12	0.002	0.005	0.2
Podophyllotoxin	0.51	0.012	0.005	4.0
Desoxypodophyllotoxin	0.54	0.005	0.015	—
β-Peltatin-A-methyl ether	0.57	0.128	—	3.0
4′-Demethylpodophyllotoxin	0.65	0.018	0.021	—
Colchicine	1.10	0.018	0.004	10.0
Podophyllic acid-2-ethylhydrazide	4.50	—	—	—
Anhydropodophyllol	5.20	25.000	—	20.0
Picropodophyllin	10.10	0.435	> 1.21	—
Epipodophyllotoxin	12.10	0.082	0.145	—
Podophyllotoxin-4,6-*O*-benzylidene-β-D-glucoside	39.50	5.720	—	—
Podophyllotoxin-β-D-glucopyranoside	180.00	27.800	0.176	—
β-Peltatin-β-D-glucopyranoside	360.00	1.740	0.26	> 1000.0
Picropodophyllic acid	> 1000.00	—	6.36	—

[*] Comparison of dissociation constants for drug-tubulin interaction (K_d) with effectiveness of compounds in assays related to inhibition of tumor growth. With the podophyllotoxin analogs, these constants were measured indirectly as inhibition constants for colchicine binding.

[†] Measured by in vitro tubulin-binding assay (7).

[‡] Measured in tissue culture by Stähelin (14) and unpublished results.

[§] Measured by a single injection to animals based on body weight, according to Leiter et al (15) as reported in ref 16.

[¶] Measured on cells in culture as described previously (17,18) and unpublished results.

comparing the effectiveness of the in vitro and in vivo tumor assays ($P \leq 0.05$). Because the precise effective dose is unknown, picropodophyllotoxin was omitted in the analysis of the in vivo tumor-damaging dose. Thus, there is a strong positive correlation between the effectiveness of these drugs as antineoplastic agents and their affinity for tubulin.

Other investigations support the pattern of drug response displayed in table 1. For example, β-peltatin-A-methyl ether, from its position in table 1, would be expected to produce tumor regression in vivo. Although this drug was not examined with the 37 sarcoma assay, Bianchi et al (19) have reported it effective against the Walker 256 carcinosarcoma in vivo at a moderately low dose. Ten percent test/control activity was observed at 0.03 μmol/g of body weight. Another trend apparent from table 1 is that the three glucosidic podophyllotoxin analogs are among the least active compounds in all assays. This fact correlates well with the low activity of glycosylated colchicine analogs in tubulin-binding assays and the damage to 37 sarcoma (6). In addition, glucosidic podophyllotoxin analogs other than those listed here have been investigated as inhibitors of tumor cell growth and were found to be as much as 10^2–10^4 times less effective than the corresponding aglucones (20).

The compounds tested in these assays differ greatly in lipid solubility. Since the more hydrophobic compounds are the most active in virtually all assays, it is possible that the correlation of the tubulin-binding assay and the biologic assays is a fortuitous one based on the fact that lipid-soluble compounds are more active in both types of assays for independent reasons. This hypothesis cannot be ruled out. However, the behavior of the compound, picropodophyllin, a stereoisomer of podophyllotoxin, suggests that the various assay methods discriminate among compounds on some criterion other than lipid solubility. The stereoisomers should not differ in lipid solubility, yet, in both the tubulin-binding assay and the in vitro and in vivo tumor growth assays, picropodophyllin is at least 20-fold less active than podophyllotoxin. Thus, we may hypothesize that the activity of the various compounds in these assays reflects their affinities for a specific binding site.

A comparison of the effective dose of various compounds in each assay reveals an interesting facet of the activity of tubulin-binding drugs, ie, that many of these compounds inhibit tumor growth in vitro at levels far below the dissociation constant for the drug-tubulin interaction. In the case of β-peltatin for example, the growth of mastocytoma cells in vitro is inhibited 50% at a drug concentration (0.128 μM) which corresponds to $< 2\%$ of the cell tubulin molecules bound to the drug, calculated from the K_d in column 1. It is a common feature of tubulin-binding drugs that polymerization of tubulin in vitro and in vivo is inhibited by substoichiometric levels of these compounds (21–23). Assuming that mouse-brain and mouse-tumor cell tubulin are equally sensitive to podophyllotoxin, the results in table 1 extend this phenomenon to several analogs of podophyllotoxin. Substoichiometric inhibition of microtubule polymerization has been shown to be due to the addition of a drug-tubulin complex to the end of a growing microtubule which prevents further addition (24).

Many of the active compounds in table 1 have merited clinical trials as cancer chemotherapeutic agents (25). However, since those compounds with high affinity for tubulin often produce noxious side effects (11,20), they have not been preferred by clinicians over the water-soluble glucosidic analogs. It is perhaps not surprising that tubulin-binding drugs have effects other than the inhibition of cell division since a wide variety of physiologic processes, including axioplasmic transport (26) and insulin release (27), require microtubules and are blocked by drugs which inhibit the polymerization of microtubules.

Recent clinical investigations have centered on a second class of podophyllotoxin analogs other than those active as tubulin-binding agents (28,29). These compounds, exemplified by VP-16-213, contain glucosidic residues and do not inhibit the polymerization of microtubules in vitro (9). They characteristically arrest cell division at the G_1 stage of the cell cycle while podophyllotoxin blocks at metaphase (30–32). Evidence indicates that VP-16-213 may produce single-strand breaks in mammalian DNA (10). These data suggest that some podophyllotoxin analogs block cell division in a manner which does not involve microtubules.

Of what value, then, are in vitro tubulin-binding assays in the development of effective chemotherapeutic agents? These assays are rapid and inexpensive and will detect compounds inhibiting microtubule polymerization by binding at the colchicine binding site. Obviously, the clinical evaluation of drugs must include such factors as intestinal absorption, detoxification, and possible noxious side effects which are beyond the scope of a drug-binding assay. Yet, the correlations displayed in table 1 suggest that tubulin-binding assays may be one useful step in the screening of podophyllotoxin-like compounds.

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Phase I Clinical Studies With Gallium Nitrate [1,2]

Agop Y. Bedikian,* Manuel Valdivieso, Gerald P. Bodey, Michael A. Burgess, Robert S. Benjamin, Stephen Hall, and Emil J Freireich [3]

SUMMARY

Gallium nitrate, an anhydrous salt of gallium, was found to have significant antitumor activity in animal tumor systems. A phase I clinical study of gallium nitrate was conducted in 42 patients with various types of solid tumors to evaluate its toxic effects and efficacy in man. The initial dose of 15 mg/m²/day × 3 days repeated at 2-week intervals was progressively increased to a maximum daily dose of 1350 mg/m². Tumor regression ≥ 50% was observed in one patient with lymphoma. Disease stabilization lasting ≥ 4 weeks was seen in ten patients. There was no correlation between abnormal pretreatment gallium scans and tumor response to gallium nitrate. Impairment of renal function was dose-limiting and was manifested mainly by the occurrence of proteinuria and azotemia. Renal toxicity was more common at daily doses > 300 mg/m² and appeared to be cumulative. Other toxic effects were nausea and vomiting, hearing loss, anemia, mucositis, metallic taste in the mouth, and lethargy. Hematologic toxicity was minimal and was manifested mainly as anemia, which appeared to be dose-dependent. The administration of gallium nitrate was associated with significant leukocytosis secondary to granulocytosis. No significant changes in the absolute lymphocyte or platelet counts were noticed as a result of treatment with gallium nitrate. Doses of 300 mg/m²/day × 3 days every 2 weeks were well-tolerated. The lack of significant myelosuppressive toxicity makes this compound attractive for further clinical studies.

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Gallium nitrate is an anhydrous salt produced by the reaction of nitric acid and gallium, a naturally occurring heavy metal. It has shown substantial growth inhibition (> 90%) of sc transplanted M-8 fibrosarcoma, 755 adenocarcinoma, P1795 lymphosarcoma, and Walker 256 carcinosarcoma (1). However, it is inactive against L1210, K1964, and P388 leukemias. In beagle dogs it produces anorexia, mucositis, diarrhea, leukocytosis, azotemia, and hepatocellular damage (2,3). The minimal toxic dose in beagle dogs is 0.32 mg/kg/day × 5 days (6.3 mg/m²).

A related compound, gallium citrate, has been found to concentrate in animal (4) and human (5–8) malignant neoplasms. Because this may also occur with gallium nitrate and because of its antitumor activity in animal tumor systems, a phase I clinical study was undertaken. The purpose of this initial study was to evaluate the efficacy and toxicity of gallium nitrate administered iv in 3-day courses.

MATERIALS AND METHODS

Adult cancer patients admitted to the Department of Developmental Therapeutics of the University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, were the subjects of this study. All patients had histologic proof of metastatic cancer and were no longer responding to

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[3] Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

**Reprint requests to:* Agop Y. Bedikian, MD, Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, Tex 77030.

conventional treatment for their tumors. Requirements for entry in the study included recovery from toxicity of previous therapy, normal blood cell counts, and normal results on renal and liver function tests. The starting dose of gallium nitrate was 15 mg/m²/day × 3 days. Subsequently, the dose was escalated by 50% if no toxicity was observed. If toxicity was observed, the dose was reduced by 50%. Courses of therapy were repeated at 2-week intervals. The drug was dissolved in 100 ml of 5% dextrose solution and infused iv for 15–30 minutes. All toxic effects noticed during or between courses of therapy were recorded.

Cbc counts were performed twice weekly; urinalysis and renal and liver function studies were repeated at least once weekly or prior to each course of therapy. Appropriate radiologic examinations as well as radioisotope-scanning procedures were usually performed prior to treatment and after two to three courses of treatment. Tumor measurements were recorded in centimeters, the longest diameter and its perpendicular applied at the widest portion of the tumor. For lung lesions, the measurements were made using roentgenograms. A complete response was defined as the disappearance of all evidence of active tumor with the patient free of all symptoms for at least 4 weeks. A partial response was defined as a $\geq 50\%$ decrease in the sum of the product of the largest diameters of the lesions. The disease was considered stabilized when there was no further growth of a previously rapidly growing tumor for at least 4 weeks or when there was a response which was less than a partial remission. Patients were considered to have progressed when there was an unequivocal increase of at least 50% in the size of any measured lesion. The development of new lesions also constituted increasing disease.

RESULTS

Forty-two adult patients with a variety of cancers who received gallium nitrate were entered in the study; their characteristics are summarized in table 1.

The dose levels of gallium nitrate used in the patients in this study ranged from 15 to 1350 mg/m²/day × 3 days (table 2). A total of 117 courses of therapy were administered. Patients were entered at the initial dose level and subsequent dose escalations were made if no significant toxicity was seen.

TABLE 1.—Patient characteristics

Total No. of patients	42
Age in yrs	
Median	48
Range	20–74
Sex	
Men	22
Women	20
No. of patients with diagnosis of—	
Malignant melanoma	19
Adenocarcinoma of lung	6
Colorectal carcinoma	5
Sarcoma	2
Breast carcinoma	2
Others*	8

*1 patient each with epidermoid carcinoma of the lung, thyroid carcinoma, Hodgkin's disease, lymphoma, hypernephroma, mucoepidermoid carcinoma, bladder carcinoma, and chordoma.

The antitumor efficacy of gallium nitrate was minimal. Tumor regression of $> 50\%$ was observed in mediastinal lymph nodes in one patient with diffuse lymphoma (mixed cell type) at a dose level of 600 mg/m²/day. Stabilization of disease in patients who were progressing rapidly with other regimens occurred at daily doses ranging from 35 to 900 mg/m². Included were three patients with malignant melanoma, two with adenocarcinoma of the lung, and one each with Hodgkin's disease and cancers of the colon, thyroid, and bladder. The duration of disease stabilization ranged from 8 to 20 weeks. The remaining patients had tumor progression.

The common toxic effects encountered in this study, as well as their relationship to the dose of gallium nitrate administered, are presented in table 3. Renal toxicity was the most frequent side effect and was dose-limiting; it was mild at doses of < 300 mg/m²/day and was manifested mainly in the form of proteinuria. The renal toxicity was dose-dependent, characterized by increased severity and frequency of proteinuria with doses > 300 mg/m² and with repeated courses. However, proteinuria > 500 mg/24 hours was rare. Acute renal failure, as mani-

TABLE 2.—Gallium nitrate phase I dose escalation

Dose level (mg/m²/day × 3)	No. of patients entered		
	Initial	Subsequent	Total
15	3	3	6
23	7	3	10
35	5	7	12
50	4	6	10
75	4	5	9
100	3	8	11
150	2	9	11
200	—	10	10
300	5	8	13
450	5	7	12
600	4	3	7
900	—	4	4
1350	—	2	2

TABLE 3.—Nonhematologic toxic effects of gallium nitrate related to dose

Dose level (mg/m²/day × 3)	No. of patients treated	% of patients with—			
		Proteinuria	Azotemia	Nausea and vomiting	Hearing loss
15–200	79	44	—	13	—
300	13	33	15	23	—
450	12	40	17	17	—
600	7	50	57	57	—
900	4	100	50	75	25
1350	2	100	50	100	100

fested by a sudden development of anuria associated with azotemia, hyperkalemia, vomiting, and change in sensorium, occurred in ten patients during 11 of 38 courses given at doses ≥ 300 mg/m²/day; it occurred during the first course of gallium nitrate in three patients and during subsequent courses in the remaining patients. One patient developed renal failure following a course associated with transient azotemia. In six of the 11 episodes, in the early stages renal failure was exhibited by proteinuria, hematuria, hypocalcemia, hyponatremia, and hyperchloremic acidosis in various combinations, followed by the development of anuria, azotemia, and hyperkalemia. In five of 11 episodes there was recovery of normal renal function upon discontinuation of treatment with gallium nitrate and institution of supportive treatment. However, four of the six other occurrences of acute renal failure were fatal, while only partial recovery of renal function occurred following the remaining two episodes. An autopsy examination of the kidneys of one of the patients who died as a result of this toxicity showed patchy areas of hyalinization, partial to complete obliteration of glomeruli, and desquamation of the lining epithelial cells into tubular lumen. Only one of the ten patients with acute renal failure had received prior treatment with *cis*-dichlorodiammineplatinum(II).

The hematologic toxicity of gallium nitrate was mild and was manifested mainly by a drop in hemoglobin level. Anemia, ie, a 3-g/100 ml drop in the hemoglobin level over a 2-week period without evidence of bleeding or hemolysis, occurred in 16 patients during 22 courses of chemotherapy. The frequency of development of significant anemia was dose-related. Eighteen percent of the courses of gallium nitrate given at dose levels between 15 and 300 mg/m²/day were associated with the development of anemia requiring blood transfusions. The corresponding number for courses given at dose levels of ≥ 450 mg/m²/day was 63%. Thrombocytopenia (platelet count $\leq 150{,}000/\text{mm}^3$) occurred during 13 of 84 (15%) evaluable courses. It was more frequent at dose levels of ≥ 450 mg/m²/day. Six of the eight courses with thrombocytopenia occurring at the latter dose levels were in patients who developed concomitant renal failure. More frequently, however, the administration of gallium nitrate was associated with significant elevation of the mean leukocyte count. This increase was mainly due to the increase in the mean granulocyte count (table 4). There was no significant change in the mean absolute lymphocyte or platelet counts as a result of treatment with gallium nitrate. Neutrophilia was seen at all dose levels but more frequently at higher dose levels.

Gastrointestinal toxicity characterized by nausea, vomiting, and diarrhea was mild. The frequency of nausea and vomiting was dose-related (table 3). Stomatitis and conjunctivitis were usually mild and infrequent. Mild sensorineural hearing loss was seen in three patients at doses ≥ 900 mg/m²/day; clinically, it was manifested by tinnitus and decreased hearing, and a high frequency hearing loss was detected on audiograms of two of the three patients who had this test. Two patients developed a metallic taste in the mouth during four courses of therapy. Two patients experienced transient lethargy during three courses and one patient developed a maculopapular rash.

A correlation of response to gallium nitrate with the results of pretreatment gallium scans was attempted in 16 patients who had undergone this test. Thirteen of these patients had abnormal gallium scans and six of these had tumor regression or disease stabilization as a result of treatment with gallium nitrate. Three patients had normal gallium scans; two of these had disease stabilization and the remaining patient had objective tumor regression which was less than a partial remission. There appears to be no correlation between the tumor uptake of gallium-67 and the response to gallium nitrate.

TABLE 4.—Hematologic toxic effects of gallium nitrate related to dose

Dose level (mg/m^2/day × 3)		Mean cell counts* (× 10^3/mm^3)					
		Wbcs		Polymorphonuclear neutrophils		Platelets	
15–75	Pretreatment	6.5		4.6		253	
	During treatment	7.3		5.8		276	
100–200	Pretreatment	5.9		3.9		185	
	During treatment	7.6		5.9		258	
300–1350	Pretreatment	5.8		4.3		290	
	During treatment	7.1		5.7		288	
Overall	Pretreatment	7.1	($P < 0.001$,	5.1	($P < 0.001$,	261	($P = 0.2$,
	During treatment	8.0	SD = 1.9)†	6.2	SD = 1.7)†	277	SD = 89.7)†

*Pretreatment = cell counts obtained immediately before the 1st course of treatment with gallium nitrate. During treatment = cell counts obtained throughout the treatment with gallium nitrate.

†Determined by Student's t test.

DISCUSSION

Since the discovery of accumulation of radioactive gallium in proliferating tissues in 1950 (9), this heavy metal has undergone extensive investigation to determine its utility in tumor localization. At present, it is well-established that gallium-67 is concentrated in a variety of animal (4) and human (5–8) tumors. The exact mechanism of accumulation in tumor cells is still unknown; however, experimental evidence indicates that a competitive binding of gallium-67 to the binding sites of calcium and magnesium cations, rather than a metabolic process, is involved (10). The greater availability of calcium and magnesium cation binding sites in proliferating and growing cells (11–13) than in normal tissue might account for the observed differences in gallium-67 uptake by neoplastic and normal tissues (14). The observation that gallium nitrate has significant antitumor activity in various experimental systems (1) has made it an attractive agent for trials in patients with neoplastic diseases.

The antitumor efficacy of gallium nitrate in the present study was very limited. There was no correlation between uptake of gallium-67 by the tumor, as demonstrated by pretreatment gallium scan, and response to gallium nitrate. The biologic effect of gallium nitrate was manifested mainly by the development of anemia, leukocytosis, and renal and gastrointestinal toxicity. Anemia and renal toxicity were dose-related. The latter was clinically shown by glomerular and tubular dysfunction which, in several cases, resulted in acute renal failure. Kidney toxicity (in particular, renal tubular damage) has been described in mice treated with gallium nitrate (2).

The hematologic effects of gallium nitrate were manifested mainly by anemia and leukocytosis. The latter was mainly due to granulocytosis and was seen at all dose levels (table 4). There was no significant change in the absolute lymphocyte and platelet counts. This effect of gallium nitrate (2) appears to be similar to that seen with lithium in experimental (15) and human (16–19) studies. Bone marrow hyperplasia and myelopoiesis in the liver has been described to occur in mice treated with indium (2), a group IIIa metal periodically related to gallium nitrate. We could not find reports about gallium-induced granulocytosis in man.

The antitumor efficacy of gallium nitrate can be established only by studying a larger number of patients. The results of this study suggest that a dose of 300 mg/m^2/day × 3 days every 2 weeks is well-tolerated. The absence of myelosuppressive toxicity during our study makes this compound attractive for further clinical studies in combination with myelosuppressive agents without a major dose reduction. Detailed therapeutic clinical and pharmacologic studies of gallium nitrate at different schedules of administration alone and in combination with other agents are indicated for a full evaluation of its efficacy.

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Variables for Predicting Serious Toxicity (Vinblastine Dose, Performance Status, and Prior Therapeutic Experience): Chemotherapy for Metastatic Testicular Cancer With *cis*-Dichlorodiammineplatinum(II), Vinblastine, and Bleomycin [1,2]

John G. Krikorian, John R. Daniels,* Byron W. Brown, Jr, and Marie S.J. Hu [3]

SUMMARY

Combination chemotherapy utilizing high-dose vinblastine, bleomycin, and *cis*-dichlorodiammineplatinum(II) (CDDP) is effective treatment for metastatic testicular cancer. Unfortunately, it is frequently associated with serious toxicity. In a series of 14 patients receiving 65 treatment cycles, several variables were examined as putative risk factors for prediction of serious toxicity. These included drug dose normalized to body weight or surface area, interval since previous cycle, prior therapeutic experience with either radiation therapy or other cytotoxic chemotherapy, and Karnofsky performance status. The strongest determinant of serious toxicity was the vinblastine dose calculated according to body weight. The next most influential prognostic variables were the performance status and a history of previous treatment with either radiation therapy or chemotherapy. Serious toxicity may be anticipated at a frequency of 40% when vinblastine is administered at a total dose of 0.36 mg/kg with bleomycin and CDDP. In our group of patients, the nephrotoxicity of CDDP appeared to be cumulative despite intensive diuresis at the time of administration. Pulmonary toxicity was not observed. Modest reductions in vinblastine dose, especially in patients with poor performance status or a history of previous radiation or other chemotherapy, will substantially lower the frequency of serious toxicity.

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Recent reports of high complete response (CR) rates and probable cure in patients with metastatic germ cell tumors of the testis have demonstrated the value of intensive chemotherapy combinations which include vinblastine, bleomycin, and *cis*-dichlorodiammineplatinum(II) (CDDP) (1–7). Since the toxic complications of current regimens may be serious, we describe them here to help physicians anticipate their appearance and to provide background in guiding the evolution of these programs. This retrospective analysis leads to the conclusion that the vinblastine dose calculated according to body weight, performance status as judged on the Karnofsky scale, and history of prior therapeutic experience with radiation or other chemotherapy programs are the dominant variables in predicting the occurrence of serious toxicity. A modest reduction in vinblastine dose, especially in patients with poor performance status and a history of prior radiation therapy or other chemotherapy, will reduce the risk of serious toxicity.

PATIENTS AND METHODS

Between June 14, 1976, and October 13, 1977, 15 patients with advanced testicular germinal neo-

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[3] Division of Oncology, Department of Medicine (*J. G. Krikorian* and *J. R. Daniels*), and Division of Biostatistics, Department of Family, Community, and Preventive Medicine (*B. W. Brown, Jr* and *M. S.-T. Hu*), Stanford University Medical Center, Calif.

**Reprint requests to:* John R. Daniels, MD, S-025, Division of Oncology, Stanford University Hospital, Stanford, Calif 94305.

TABLE 1.—Characteristics of patients

Patient	Age in yrs	Diagnosis	Prior radio-therapy	Prior chemo-therapy	Current status*
A	31	Embryonal cell carcinoma		×	Dead
B	27	Embryonal cell carcinoma	×	×	Dead
C	25	Embryonal cell carcinoma		×	Dead
D	22	Teratocarcinoma	×		Alive with disease; therapy discontinued
E	18	Endodermal sinus tumor	×	×	Dead
F	29	Embryonal cell carcinoma	×	×	Alive without disease; therapy discontinued
G	29	Embryonal cell carcinoma	×	×	Dead
H	35	Embryonal cell carcinoma	×		Alive without disease; therapy discontinued
I	25	Embryonal cell carcinoma	×	×	Dead
J	21	Embryonal cell carcinoma			Alive without disease; receiving therapy
K	23	Choriocarcinoma			Dead
L	24	Teratocarcinoma		×	Alive without disease; receiving therapy
M	35	Embryonal cell carcinoma			Alive without disease; therapy discontinued
N	30	Embryonal cell carcinoma	×	×	Alive without disease; therapy discontinued
O	28	Embryonal cell carcinoma			Alive without disease; receiving therapy

*As of Oct 1, 1977.

plasms (8) were treated with combination chemotherapy, including vinblastine, bleomycin, and CDDP, for a total of 66 treatment cycles. Patients are designated by letters and each treatment course is indicated by the addition of a subscript.

Patient characteristics are listed in table 1. Six patients had received both prior radiation therapy and chemotherapy, two had received only prior radiation therapy, and three had received only prior chemotherapy. Four patients were previously untreated.

Chemotherapy was administered according to a schedule modified from Einhorn et al (1,2): vinblastine at a dose of 7.5 mg/m^2 iv on Days 1 and 2, bleomycin at a dose of 15 units/m^2 iv on Day 2 and then weekly, and CDDP at a dose of 20 mg/m^2 iv on Days 1–5. For the 5 hours preceding the administration of CDDP, patients were hydrated by infusion with 1000 ml of 5% dextrose in $^1/_2$ normal saline iv at 200 ml/hour. Just prior to the administration of CDDP, patients received 25 g of mannitol iv and 40 mg of furosemide iv. Fluids and electrolytes were infused at a rate not < 200 ml/hour for the next 5 hours or longer, until vomiting stopped. Chemotherapy cycles were to be administered every 3–4 weeks. While the initial intent had been to discontinue all chemotherapy at the completion of eight cycles, we decided to use no more than four treatment cycles after complete disappearance of disease.

In six cycles (G_6, H_5, L_7, M_5, $O_{6,7}$) the total CDDP dose was given over 24 hours by continuous infusion. Five patients did not receive bleomycin for a total of 17 cycles because they had received the maximum allowable cumulative dose ($A_{1,2}$, F_{1-5}, G_{1-6}, H_5, J_{5-7}). Bleomycin was given at one-half dose in the L_5 cycle and at full dose in the remaining cycles.

Because of the frequent occurrence of serious toxicity and difficulties with patient compliance, drug doses were frequently individualized. Seven patients received < 100% of the calculated CDDP dose for a total of 20 cycles ($A_{1,2}$, E_{2-6}, $F_{2,4}$, $G_{5,6}$, $J_{6,7}$, $L_{3-5,8}$, $O_{2,6,7}$). In eight of these 20 cycles, CDDP was given at 85%–92% of the calculated dose.

The vinblastine dose was variable and only 19 cycles were given at full dose.

Patients were assigned a performance status on the Karnofsky scale (9) at the beginning of each treatment cycle and weekly at each interim outpatient clinic visit. Although this was to have been obtained prospectively, a performance status was assigned retrospectively when its recording had been omitted.

STATISTICAL METHODS

An endpoint of serious toxicity was defined as any toxicity following treatment requiring hospitalization for management. A multiple logistic regression (15) function was used to determine the variables that most strongly predicted the occurrence of serious toxicity (ST):

$$P(ST) = \frac{1}{1 + e^{-(A \cdot X + B \cdot Y + C \cdot Z + D)}}$$

A univariate analysis was performed to determine which drug (X) most strongly predicted the occurrence of serious toxicity. Subsequent analyses tested the dosage basis (body surface area or weight) and then other variables (Y and Z) were examined individually and simultaneously for additional prognostic value.

The best multiple logistic regression function was chosen by selecting the estimates of the constants A, B, and C that maximize the log likelihood, function (L):

$$L = \sum_{i=1}^{n_1} \log P_i + \sum_{j=1}^{n_2} \log (1 - P_j)$$

$P_i, i = 1,2 \ldots n_1$ corresponds to toxic episodes; n_1 = number of toxic episodes

$P_j, j = 1,2 \ldots n_2$ corresponds to nontoxic episodes; n_2 = number of nontoxic episodes

RESULTS

Sixty-six cycles of chemotherapy were given to 15 patients. One patient (K, table 1) with choriocarcinoma exsanguinated from a liver metastasis on the day of completing his first cycle of chemotherapy and is not evaluable. Only one patient completed the intended eight cycles of therapy.

Response

Eight of 14 evaluable patients achieved CR status, and seven are no longer receiving therapy and have no evidence of disease (range, 1–9 months). Of these seven patients, three had failed previous chemotherapy. One patient (D), previously treated only with radiation therapy, achieved CR but has equivocal signs of relapse after 6 months. Six patients have died with disease; all of these had received previous chemotherapy.

Toxicity

A total of 65 cycles of chemotherapy could be evaluated in 14 patients. There were no toxicity-associated deaths. Nausea, vomiting, and alopecia were universal. Mucositis, ileus, and urinary retention were common. Acute neuromuscular toxicity requiring analgesics, attributable to vinblastine, was observed in five patients; however, persistent neurologic damage was not seen. One patient (O) developed an unexplained seizure disorder following his fourth cycle. Seven patients required intermittent transfusions for a total of 29 units of blood. Nadir wbc and platelet counts were not uniformly measured; however, leukopenia was present when determined at the expected nadir. One patient (J) developed an abscess of the anterior abdominal wall requiring excision.

Twenty of these 65 cycles were complicated by serious toxicity, defined as requiring hospitalization for management. Table 2 lists the spectrum of this toxicity. In all but two instances, gastrointestinal complications were a prominent component. All patients were profoundly granulocytopenic and usually febrile. Thrombocytopenia was frequently present, but there was only one clinically important bleeding episode (N_1). Two patients required laparotomy.

Figure 1 shows the average leukocyte and platelet counts during the 3-week period following drug administration that led to the 20 episodes of serious toxicity. Profound leukopenia was observed between 7 and 14 days following the initiation of the cycle and was universal between Days 9 and 11. Recovery was complete by Day 21. Platelet counts fell by > 50% by Day 3, but this early fall did not predict subsequent severe thrombocytopenia (platelets < 50,000/mm^3) and suggests accelerated peripheral destruction. The most dramatic example of this early fall occurred in a nontoxic cycle (E_4) where the platelet count fell from 150,000/mm^3 at the initiation of drug therapy to 30,000/mm^3 by Day 3 with subsequent normal recovery.

The course of therapy of each patient is indicated in figure 2, which shows the complicated and uncomplicated cycles, performance status at each cycle initiation, and interval in days between cycles. The median performance status score was 90 (range, 60–100). Of note is how infrequently patients were able to comply with a 3-week interval between cycles (mean/median interval: 38/33 days). The decreasing incidence of serious toxicity with higher cycle numbers reflects dose reductions following cycles complicated by serious toxicity.

Figure 3 relates the frequency of serious toxicity to the vinblastine dose calculated according to body

TABLE 2.—Characteristics of episodes of serious toxicity

	Lowest recorded count					
Patient (cycle)	Platelets × 10^3/mm^3	Wbcs/mm^3 (% neutrophils)	Maximum recorded temperature (°C)	Bacteremia	Gastrointestinal complications	Other complications
A_1	4	0	39.2		Perirectal abscess	
B_1	18	900 (29)	39.2	*Klebsiella*	Abdominal tenderness	
C_1	90	800 (2)	39.8		Appendiceal abscess	
D_1	80	400 (0)	39.5		Rectal fissure	
E_1	90	400 (2)	39.0	*Escherichia coli*	Obstipation	Pneumonia
H_1	146	400 (1)	39.1		Ileus	Pneumonia
I_1	44	400 (0)	39.2		Ileus, hematemesis, and abdominal tenderness	
M_1	125	700 (1)	39.5		Ileus	Urinary retention
N_1	4	200 (0)	40.0		Jaundice	*E. coli* pneumonia and thrombocytopenic bleeding
A_2	32	600 (6)	40.0		Perirectal abscess	
D_2	90	600 (4)	38.4		Perineal ulcer	
I_2	68	400 (0)	39.2	*Staphylococcus aureus*	Staphylococcal mucositis	Lung abscess
L_2	86	600 (0)	40.1			
C_3	45	600 (0)	38.7		Perineal ulcer	
D_3	68	700 (1)	39.6		Ileus	
G_3	43	500 (0)	37.9			Epididymitis
D_4	95	1200 (4)	39.3		Perforated ileum and peritonitis	
O_4	80	1300 (4)	39.3		Abdominal pain and nausea and vomiting	Seizures and urinary retention
E_5	*	600 (*)	*		Appendicitis and laparotomy	Upper respiratory tract infection
L_7	51	1000 (0)	39.8		Mucositis	

*Information not available; patient hospitalized elsewhere.

surface area and according to body weight at the initiation of each cycle. The incidence of serious toxicity increased with the vinblastine dose calculated according to either body surface area or body weight. However, the dose of vinblastine calculated according to body weight was better fit to a logistic regression function and more strongly predicted the occurrence of serious toxicity (table 3). The occurrence of serious toxicity rose sharply to > 0.18 mg/kg daily for 2 days.

Variables including doses of other drugs, therapeutic experience with either radiation therapy or chemotherapy prior to initiation of treatment with CDDP, vinblastine, and bleomycin, interval since the previous treatment cycle, and performance status were examined for potential prognostic significance. Performance status and prior therapeutic experience contributed additional prognostic significance (fig 4 and table 3). The best fit was obtained (minimum log likelihood) when all three factors were considered: vinblastine dose on a weight basis, performance status, and history of prior treatment (table 3 and fig 4 A and B). The interval since previous cycle and the cycle of chemotherapy were of no added value for predicting serious toxicity. Weight and Karnofsky status were more highly correlated ($r = 0.50$ and $P < 0.01$) than were body surface area and Karnofsky status, accounting for the stronger incremental influence of Karnofsky status when vinblastine doses were computed according to body surface area rather than weight.

Renal function was monitored before each treatment cycle with serum creatinine and BUN determinations. Twelve of the 14 patients had initial measurements of creatinine clearance prior to the initiation of the drug program. Of these 12, ten had subsequent determinations of creatinine clearance. Figure 5 shows the average change in serum creat-

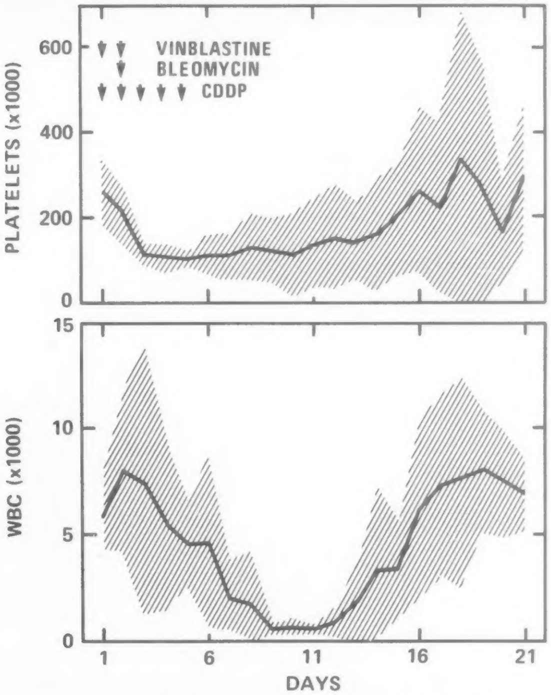

FIGURE 1.—Mean platelet and wbc counts during the 3 wks following the initiation of cycles complicated by serious toxicity. Shaded areas indicate the SD of the mean values.

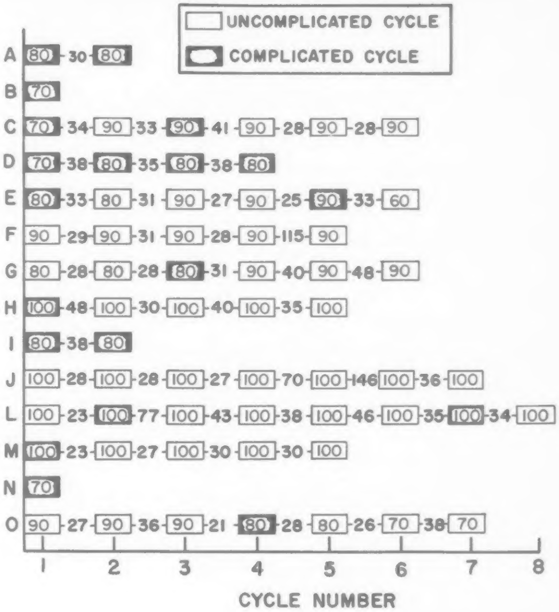

FIGURE 2.—Summary of each patient's course with cycles complicated by serious toxicity. Intervals between cycles and performance status are indicated.

inine and creatinine clearance values following each cycle. Usually, values were determined at the beginning of the next cycle or 1 month after treatment. Deterioration of renal function presumably secondary to CDDP was reflected by serum creatinine which rose by 30% after five cycles and creatinine clearance which dropped to 50% of pretreatment values after five cycles of therapy. The magnitude of the decline of renal function was independent of the intervening aminoglycoside experience in those patients who were treated for fever and neutropenia. Creatinine clearance was a more sensitive indicator of the decline in renal function than was serum creatinine.

Four patients reaching the fourth cycle had both baseline and followup audiograms. Two of these patients had asymptomatic high-frequency sensorineural hearing loss.

No patient developed signs or symptoms of bleomycin pulmonary toxicity.

DISCUSSION

This study documents the serious toxicity of the widely used chemotherapy combination utilizing high-dose vinblastine, bleomycin, and CDDP. When given with bleomycin and CDDP, serious toxicity was most frequent when vinblastine was administered at a dose of ≥ 0.18 mg/kg for 2 successive days (total dose, 0.36 mg/kg). The risk of serious toxicity was also dependent on the performance status at the initiation of the treatment cycle as well as on the prior therapeutic experience with either radiation therapy or other chemotherapy programs. In a previously untreated and relatively asymptomatic population, overall drug tolerance should be improved.

Performance status assessment utilizing defined scales was introduced to the practice of oncology by Karnofsky (9). His initial ten-point scale and a related five-point system developed by the Eastern Cooperative Oncology Group are widely used. The clinical validity of performance status assessment has been established in two ways. It is an important predictor of survival in patients with lung cancer (10) and of response to chemotherapy in colon cancer (11). In the study reported here, the clinical application of performance status assessment has been extended to the prediction of toxicity. It is remarkable that this relationship was demonstrable over a relatively narrow range at the upper limits of the scale. It remains for future studies to deter-

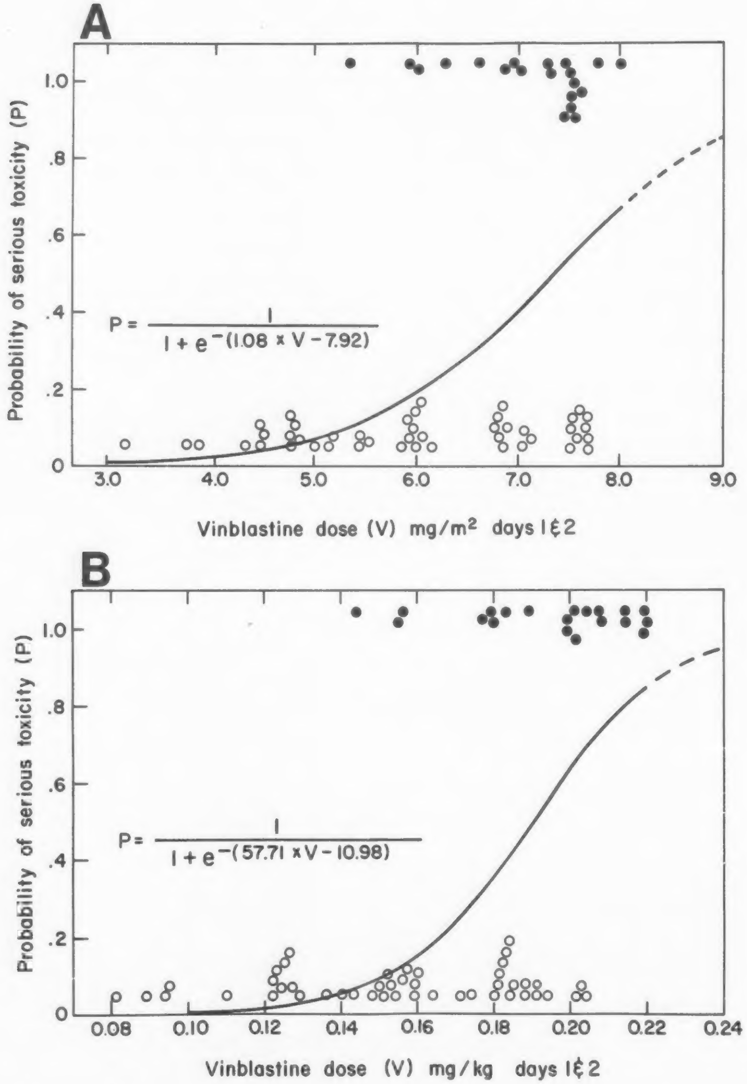

FIGURE 3.—Relationship between the probability of serious toxicity and the vinblastine dose expressed both as mg/kg (A) and mg/m^2 (B). Uncomplicated cycles (○) and complicated cycles (●).

$$P = \frac{1}{1 + e^{-(52.64 \times V - 0.046 \times K + 1.28 \times T - 6.95)}}$$

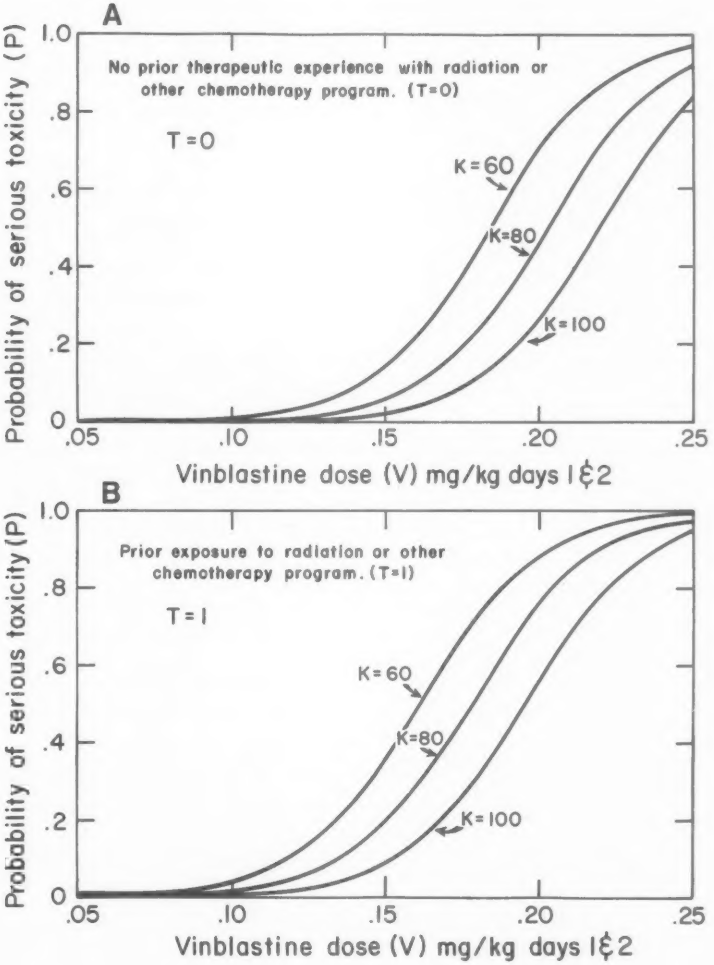

FIGURE 4.—Probability of serious toxicity. Influence of Karnofsky performance status on vinblastine dose-effect relationship. (A) Patients without prior therapeutic experience; (B) patients with prior therapeutic experience with radiation or other chemotherapy program.

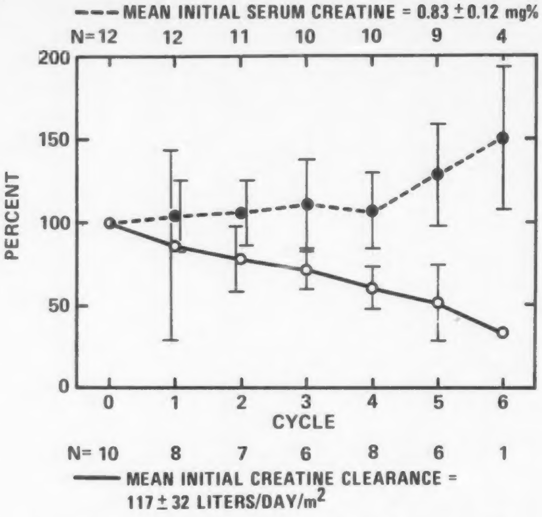

FIGURE 5.—Mean relative change in serum creatinine and creatinine clearance following each cycle.

mine whether performance scale assessment will predict for chemotherapy toxicity in other clinical settings. These scales have not been formally evaluated for linearity or observer variability. One would expect improved scales to be even more useful.

Body surface area more accurately predicts drug tolerance across several species than does body weight (12,13). This has led in recent years to a wide acceptance of surface area as the basis for calculation of drug dose in man. The relative merits of weight and surface area are not independently established within adult populations and may in fact differ from drug to drug depending on the relative importance of volume of distribution (better predicted by weight) and of clearance mechanisms (better predicted by surface area). The vinblastine dose normalized to body weight more accurately predicted the occurrence of serious toxicity. Using surface area to compute delivered dose results in lighter patients receiving more and heavier patients receiving less drug than when computed on a weight basis.

The incidence of serious toxicity reported here appears comparable to that reported by Einhorn and Donahue (3) who are currently evaluating the efficacy of their regimen utilizing a 25% reduction in the 0.4-mg/kg total vinblastine dose. Although we do not know the dose-response relationship for vinblastine in testicular cancer, the therapeutic index of vinblastine probably declines above a total dose of 0.36 mg/kg since the incidence of life-threatening toxicity sharply increases. A reduction in the frequency of serious toxicity may also permit greater patient compliance with a 3–4-week interval between cycles. Based on the findings reported here, we now administer vinblastine at a dose derived from figure 4 A and B to result in an expected incidence of serious toxicity which does not exceed 10% of courses. Thus, patients with no risk factors will receive 0.17 mg/kg of vinblastine on Days 1 and 2 with further reduction for poor performance status and for prior therapeutic experience with radiation therapy or other chemotherapy programs.

The decline in renal function appeared moderate when measured by serum creatinine or BUN; however, creatinine clearance was severely affected despite an intensive diuresis at the time of CDDP administration. Whether this nephrotoxicity is reversible awaits long-term evaluation. Similar findings have been reported (3,14).

Ototoxicity was not clinically apparent; however,

TABLE 3.—Probability of serious toxicity = $[1 + \exp - (A \cdot X + B \cdot Y + C \cdot Z + D)]^{-1}$

X*	Y	Z	A	Student's t test	B	Student's t test	C	Student's t test	D	Student's t test	Log likelihood
V	—	—	0.286	2.32	—	—	—	—	–4.286	–1.75	–37.1
V/m²	—	—	1.080	3.12	—	—	—	—	–7.92	–1.39	–32.9
V/kg	—	—	57.71	3.70	—	—	—	—	–10.98	–1.32	–27.6
V	K†	—	0.400	2.75	–0.104	–3.17	—	—	3.445	0.44	–30.9
V/m²	K	—	1.243	3.06	–0.096	–2.91	—	—	–0.530	–0.04	–27.9
V/kg	K	—	53.21	3.32	–0.061	–1.181	—	—	–4.757	–0.26	–25.8
V/m²	—	T‡	1.36	3.45	—	—	2.48	2.72	–11.68	–1.31	–27.8
V/kg	—	T	56.99	3.59	—	—	1.68	1.86	–12.14	–1.28	–25.5
V/m²	K	T	1.36	3.22	–0.076	–2.16	1.88	2.07	–4.41	–0.25	–25.3
V/kg	K	T	52.64	3.25	–0.046	–1.25	1.28	1.39	–6.95	–0.29	–24.8

*V = vinblastine dose/day.

†K = Karnofsky performance status.

‡T = prior therapeutic experience with radiation or other chemotherapy programs.

it was infrequently monitored with audiograms. Asymptomatic high-frequency sensorineural hearing loss was observed in two patients.

Modest reductions in vinblastine dose in this treatment program may substantially reduce the risk for serious toxicity, possibly without having a similar impact on its efficacy.

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Probenecid Inhibition of Methotrexate-Cerebrospinal Fluid Pharmacokinetics in Dogs [1]

A. Ramu,[2] J. E. Fusner,[2,*] T. Blaschke,[3] and D. L. Glaubiger [2,4]

SUMMARY

Cerebrospinal fluid (CSF) and plasma concentrations of methotrexate (MTX) were followed in dogs for 72 hours after intracisternal injection of MTX with and without probenecid pretreatment. CSF levels declined as a biexponential function of time. Probenecid pretreatment of the animals prolonged the second phase half-disappearance time of MTX from 5.20 ± 0.89 to 7.086 ± 0.23 hours (mean ± SD). The peak mean plasma concentration of MTX was lower in the presence of probenecid. Also, the rate of decline of plasma MTX concentrations was slower after treatment with probenecid, with mean half-disappearance times of 7.60 ± 0.77 and 11.32 ± 1.08 hours. These CSF and plasma data support the proposal that probenecid inhibits the transfer of MTX from CSF to blood.

[Cancer Treat Rep 62:1465–1470, 1978]

One of the more widely used and effective regimens for the prophylaxis and treatment of meningeal leukemia is the intrathecal administration of methotrexate (MTX). Several different dose schedules are currently in use as well as administration in conjunction with other agents. Toxic effects of varying severity have been noted (1). Although the relationship between cerebrospinal fluid (CSF)-MTX concentration and efficacy and toxicity are not well-understood, certain facts are known. The cytotoxic action of MTX requires the presence of free intracellular drug above a specific threshold level for each tissue. The effect of MTX on a target tissue is primarily a function of duration of exposure to suprathreshold concentrations of drug, rather than the peak levels of drug achieved (2). Studies on leukemic lymphoblasts have indicated that the minimum effective therapeutic MTX concentration for these cells is approximately 10^{-6}M (3). The therapeutic interval after intrathecal administration of MTX would, therefore, be the time that CSF-MTX concentrations remained $> 10^{-6}$M. After intrathecal administration of 12 mg/m² of MTX in man via lumbar injection, CSF-MTX concentrations fall from 2×10^{-3} to $< 10^{-6}$M within 24 hours (4).

Since there are data to suggest that more protracted exposure to therapeutic MTX concentrations is more efficacious (5), methods of reducing the rate of decline of CSF-MTX concentration following intrathecal instillation are of interest. We have investigated the possible utility of probenecid in slowing the rate of decline of CSF-MTX concentration.

Several lines of experimental evidence support the idea that probenecid administered systemically can reduce the rate of MTX efflux from CSF. A number of studies have shown that probenecid inhibits active transport of organic acids out of the CSF (6,7). Rubin et al (8) found that the choroid plexus of the rabbit could extract MTX from a medium against a concentration gradient, suggesting active transport of MTX. In another study in rabbits, Spector (9) showed that CSF-MTX levels

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[2] Division of Cancer Treatment, National Cancer Institute, Bethesda, Md.

[3] Division of Clinical Pharmacology, Department of Medicine, Stanford University Medical Center, Calif. Recipient of a Faculty Development Award in Clinical Pharmacology from the Pharmaceutical Manufacturers Association Foundation.

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*_Reprint requests to:_ June E. Fusner, MD, Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute, Bldg 10, Rm 3B06, National Institutes of Health, Bethesda, Md 20014.

measured 4 hours subsequent to administration were elevated by pretreatment with ip probenecid. In the present study, larger animals (dogs) in which serial CSF- and plasma-MTX measurements could be made were used and MTX kinetics were determined with and without continuous probenecid pretreatment.

MATERIALS AND METHODS

Eight American foxhounds, weighing 20.4–28.5 kg, were anesthetized with 30 mg/kg iv of pentobarbitone sodium. Additional doses were given as needed for the first 13 hours. For later CSF samplings the dogs were re-anesthetized for short periods with 8.5 mg/kg of thiamyl sodium iv (Surital, Parke-Davis). Percutaneous cisternal puncture was performed using a 20-gauge spinal needle. A slow infusion of normal saline was begun in a hindlimb vein, and another catheter, filled with heparin (30 units/ml), was placed in a forelimb vein and used for withdrawing blood samples.

After withdrawing 1 ml of CSF (0 time sample), a dose of 0.4 mg/kg of ^{3}H-MTX, from a solution containing 20 mg/ml of MTX with a specific activity of 3.486 millicuries/mmol (prepared from MTX, Ben Venue Laboratories, Inc, and [3′,5′,9′(n)-^{3}H]MTX, 13.5 curies/mmol, Amersham/Searle), was diluted in saline to a volume of 1 ml and injected intracisternally. CSF was aspirated into a syringe (1 ml) three times to facilitate mixing. CSF samples of 1.50 μl were withdrawn at 0.5, 1, 2, 3, 5, 7, 10, and 13 hours. At 24, 31, and 48 hours, 1 ml of CSF was withdrawn and at 55 and 72 hours, 2 ml was withdrawn. Blood samples were taken at 0, 15, 30, 45, 60, 75, and 90 minutes, and thereafter at 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 10, 13, 24, 31, 48, 55, and 72 hours. The blood samples were centrifuged, and the separated plasma samples were stored frozen until being analyzed for MTX concentration.

Four of the dogs, allocated randomly, were treated with 1 g of probenecid every 8 hours orally for 72 hours prior to the MTX administration and throughout the experiment (when under anesthesia, the drug was given iv). CSF samples (in duplicate) were placed in counting vials containing 15 ml of Aquasol (New England Nuclear Corp). The samples were counted to at least 5000 counts using a Packard Liquid Scintillation Spectrometer (Model 3390).

CSF and plasma concentrations were calculated by a spectrophotometric enzyme inhibition assay using *Lactobacillus casei* (10). The CSF-MTX concentrations were also calculated from the measured radioactivity in the samples. [Although ^{3}H-MTX metabolites appeared in rats and mice, most likely produced by intestinal bacteria (11,12), none are found in dogs (13).]

RESULTS

CSF-MTX levels following a single intracisternal injection of MTX are shown in figure 1. Concentrations were determined by monitoring radioactivity of tritiated drug and by assaying aliquots of the same samples using a spectrophotometric enzyme inhibition method. As shown in figure 1, results from both measurement techniques are similar, suggesting that there is no significant metabolism of MTX in the CSF. The MTX disappearance curve over 72 hours following administration fits a biexponential decay, with the first (α) phase occurring between 0 and 7 hours and the second (β) phase occurring between 7 and 72 hours, the latest time point measured. The β-phase exponential decay slope was calculated using linear regression analysis and a least-squares fitting technique. Correlation coefficients for exponential slopes thus obtained were between 0.91 and 0.996. The α-phase exponential decay slope was then calculated using a stripping technique (14). The data were also analyzed using a nonlinear least-squares regression program (MLAB) developed for the DEC PDP 11 Computer (15). The results from both methods were similar.

MTX decay following intracisternal injection subsequent to pretreatment with systemic probenecid is shown in figure 2. Levels were determined using both radiolabeled drug and an enzyme inhibition assay and, as before, no difference was noted. A biexponential decay curve was again observed and analyzed by the methods outlined above. Both analysis techniques again produced similar results. Mean calculated intercepts and half-disappearance time values for the two exponential terms, both with and without probenecid pretreatment, are shown in table 1. MTX concentrations were determined by the spectrophotometric method and analyzed by the stripping technique.

From the mean values for intercepts and slopes of the two phases, the mean CSF-MTX clearances [mean clearance = dose/([first intercept × $t_{1/2\alpha}$/0.693] + [second intercept × $t_{1/2\beta}$/0.693])] were calculated (16). The mean CSF-MTX clearance was 3.15 ml/hour without probenecid and 2.42 ml/hour with probenecid treatment.

Since there was no significant difference between the intercepts of the second exponential phases, the extrapolated volume of distribution (Vd_{ext} = dose/second phase intercept) could be calculated from the mean values of dose and second phase intercept and was found to be 49.1 ml.

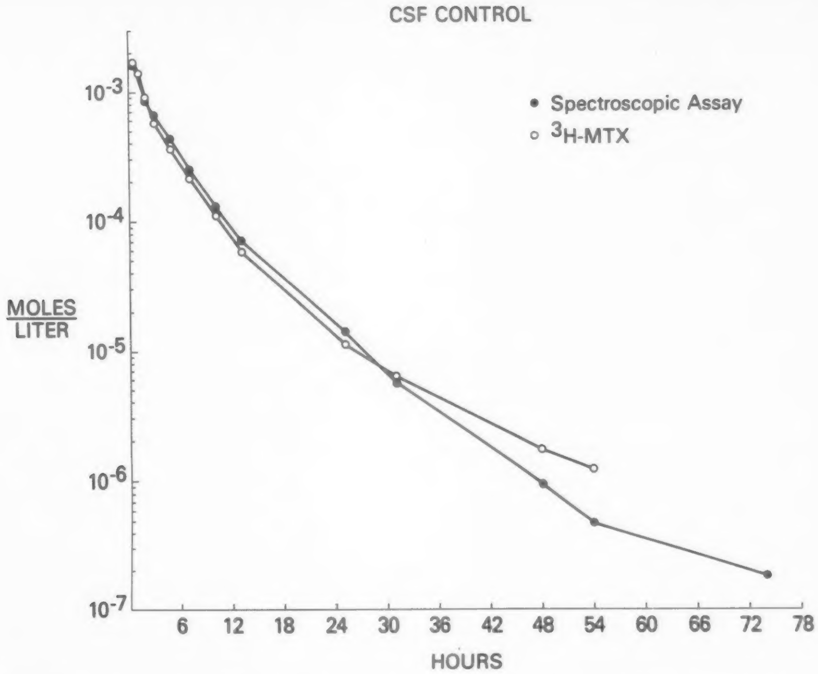

FIGURE 1.—CSF-MTX disappearance curve following intracisternal injection of 0.4 mg/kg of MTX. Concentrations were determined by (*a*) monitoring radioactivity of tritiated drug and by (*b*) assaying aliquots of the same samples using a spectrophotometric enzyme inhibition method, and no difference was noted.

Using the equation describing biexponential decay

$$C_t = Axe^{\frac{-0.693.}{t_{1/2\alpha}}t} + Bxe^{\frac{-0.693.}{t_{1/2\beta}}t}$$

(where C_t = CSF-MTX concentration at time t, A = intercept of the first exponential phase, $t_{1/2\alpha}$ = half-disappearance time of the first phase, B = intercept of the second exponential phase, and $t_{1/2\beta}$ = half-disappearance time of the second phase), it was calculated that following intracisternal injection of 0.4 mg/kg of MTX, the mean concentration would be 2.10×10^{-3}M at 0 time, $> 1 \times 10^{-6}$M at 45 hours, and 3.0×10^{-8}M at 72 hours. With probenecid treatment, the concentration would be 2.33×10^{-3}M at 0 time and $> 1 \times 10^{-6}$M at 62 hours.

Plasma-MTX concentration curves subsequent to intracisternal MTX injection, with and without probenecid pretreatment, are shown in figure 3. Without probenecid pretreatment, the peak mean plasma-MTX concentration was $3.44 \pm 0.86 \times 10^{-7}$M (mean ± SD). This peak level was reached 4 hours subsequent to injection. Intrathecal administration of MTX in the dogs treated with probenecid gave a significantly lower peak mean concentration of $2.05 \pm 0.36 \times 10^{-7}$M which was achieved about 5 hours after injection. A single exponential decay of plasma-MTX levels was observed after the peak level was attained. Mean calculated intercepts and half-disappearance times, both with and without probenecid pretreatment, are shown in table 2. MTX concentrations were determined by the spectrophotometric method and analyzed by the stripping technique. The mean half-disappearance time calculated with probenecid pretreatment (11.32 hours) was significantly greater than without probenecid pretreatment (7.6 hours).

DISCUSSION

CSF-MTX concentrations subsequent to the intracisternal injection of MTX follow a biexponential decay curve for at least 72 hours. The first decay half-disappearance time was 1.50 ± 0.50 hours, and no significant difference was found between MTX treatment and probenecid-MTX treatment. This result was not unexpected, since the first exponential phase most likely represents mainly distribution of

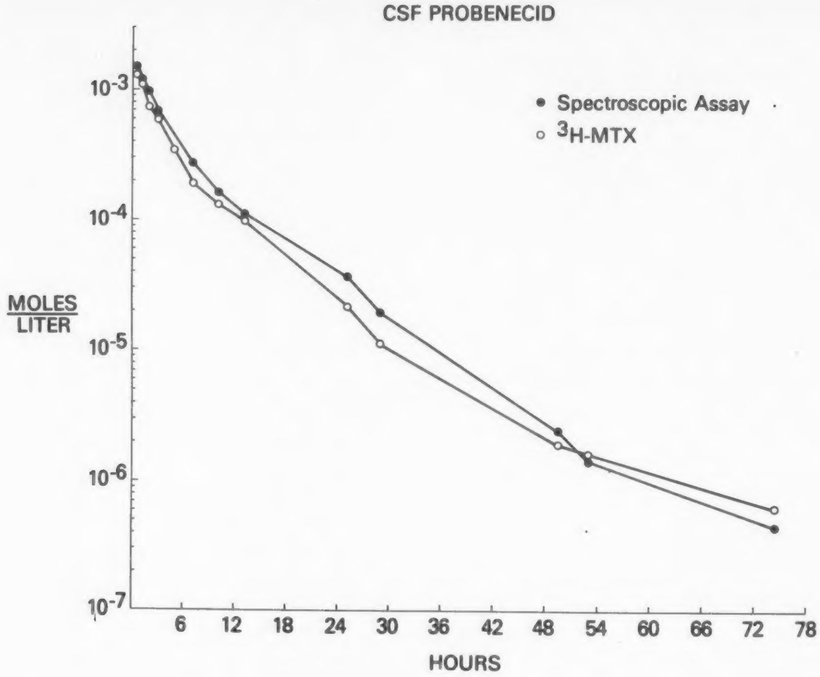

FIGURE 2.—CSF-MTX disappearance curve subsequent to intracisternal injection of 0.4 mg/kg of MTX and pretreatment with systemic probenecid (1 g q8h orally for 72 hrs prior to injection). Levels were determined using both (*a*) radiolabeled drug and (*b*) an enzyme inhibition assay, and results from both measurements were similar.

TABLE 1.—CSF kinetic parameters

	Parameter			
	α-phase		β-phase	
	Intercept (mM)	$t_{\frac{1}{2}\alpha}$* (hrs)	Intercept (mM)	$t_{\frac{1}{2}\beta}$* (hrs)
No probenecid				
Mean	1.66	1.50	0.44	5.20
SE	0.17	0.29	0.07	0.52
Probenecid				
Mean	1.88	1.61	0.45	7.09
SE	0.35	0.28	0.09	0.12

*$t_{\frac{1}{2}\alpha}$ = mean half-disappearance time of the 1st phase and $t_{\frac{1}{2}\beta}$ = mean half-disappearance time of the 2nd phase.

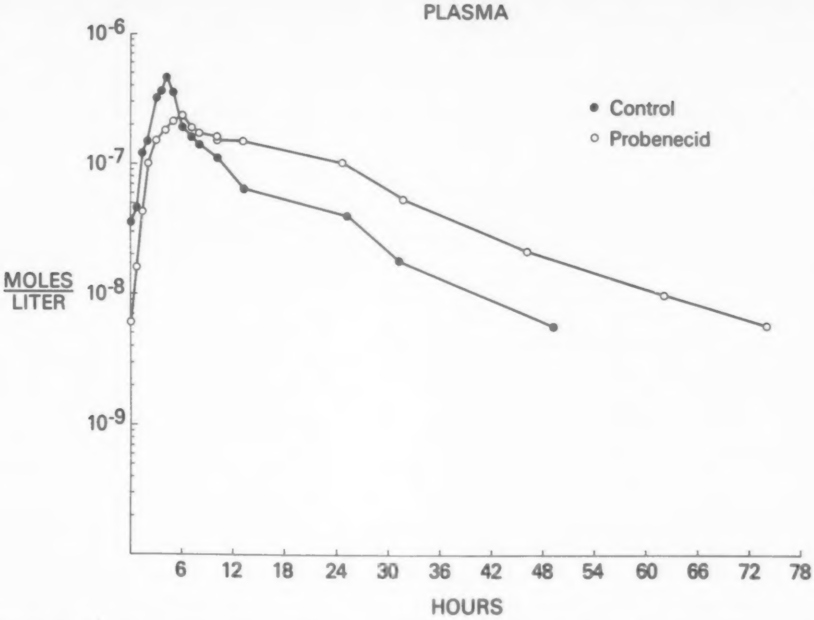

FIGURE 3.—Plasma-MTX concentration curves subsequent to intracisternal injections of 0.4 mg/kg of MTX, with and without probenecid pretreatment. Levels were determined using an enzyme inhibition assay.

TABLE 2.—Plasma kinetic parameters

	Parameter	
	Intercept (10^{-6}M)	$t_{1/2}$* (hrs)
No probenecid		
Mean	0.37	7.60
SE	0.01	0.35
Probenecid		
Mean	0.24	11.32
SE	0.03	0.54

* $t_{1/2}$ = mean half-disappearance time.

MTX into the CSF and extracellular spaces of the brain. The second exponential phase probably represents the elimination of MTX from the CSF. Since there was no significant difference in the extrapolated volumes of distribution between the two treatments, the increase in the second phase half-disappearance time during probenecid-MTX treatment reflects decreased transport of MTX out of the CSF. Indeed, the calculated mean CSF-MTX clearance was about 25% lower.

The plasma-MTX concentration data provide support for the proposal that probenecid inhibits the transfer of MTX from the CSF to the plasma. After reaching the systemic circulation, the major portion of the administered MTX is subsequently eliminated by the kidneys. The half-disappearance time of MTX in plasma is significantly shorter than that found in the probenecid-MTX portion of the study and is consistent with previous studies which show that probenecid, as well as other organic acids, interferes with the renal (17,18) and biliary (19) elimination of MTX. If probenecid affected only the plasma clearance of MTX and not its transfer to the plasma from the CSF, inhibition of the plasma clearance of MTX should result in higher maximal plasma-MTX concentrations after intrathecal administration in the portion of the study in which the dogs received probenecid. In fact, the peak plasma-MTX concentration was reduced. This could only occur if the rate of transfer of MTX from CSF to plasma was reduced or if the peripheral tissue distribution of MTX was increased. The latter possibility is much less likely and not supported by other studies of the effect of probenecid on the disposition of systemically administered MTX (17,19).

The effects of probenecid on CSF-MTX clearance might be explained in a number of ways. Probenecid may interfere with the balance of CSF production and absorption, possibly by decreasing production or increasing absorption of CSF by the choroid plexus (20) or by blocking drainage by the arachnoid villi. However, no information is available to support either of these possibilities. There may be interference with the active transport of MTX from the CSF by the choroid plexus. Although some studies suggest such a mechanism (8,9), it may not account for the entire decrease in CSF-MTX clearance.

In summary, this study shows that the therapeutic interval (ie, the time the CSF-MTX concentration is $> 10^{-6}$M) is prolonged in the probenecid-treated animals by 38%, from 45 to 62 hours.

The probenecid effect on MTX given into the CSF has now been observed in several species (rabbit, dog, and monkey) using pharmacologically accepted doses of probenecid and MTX. The effect in the rabbit has not been quantitated and the effect in the dog as described in this study is significant but not dramatic. Studies in the monkey show a greater effect by probenecid on MTX transport from CSF to plasma.[5] Since the monkey is an excellent primate model for CSF-MTX kinetics in man, probenecid inhibition of MTX transport from CSF to plasma may be clinically useful (21).

Further considerations are necessary, however, prior to utilization of these results. Probenecid may inhibit the antitumor effect of MTX and prolonged central nervous system exposure to high MTX levels may result in increased toxicity (22).

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Biology and Therapeutic Response of a Mouse Mammary Adenocarcinoma (16/C) and Its Potential as a Model for Surgical Adjuvant Chemotherapy[1,2]

T. H. Corbett,* D. P. Griswold, Jr, B. J. Roberts, J. C. Peckham, and F. M. Schabel, Jr[3,4]

SUMMARY

A mammary adenocarcinoma (16/C) was isolated and maintained in serial passage by transplantation of metastatic lung foci. This tumor originated as a spontaneous mammary adenocarcinoma in a C3H/He female mouse. It was selected as a model from > 50 mammary tumors studied because it was highly metastatic and because it responded to most of the agents reported to be active against breast cancer in women. Sc implanted 16/C tumors (in the 300–1000-mg range) metastasized to the lungs in > 75% of the mice and to the axillary lymph nodes in > 30%. This tumor has been tested for sensitivity to > 40 clinically used agents. Adriamycin was the most active single agent. Other active agents included cyclophosphamide, 5-fluorouracil, vincristine, melphalan, dibromodulcitol, maytansine, neocarzinostatin, palmO-ara-C, vinblastine, and VP-16-213. Agents most active against 40–1000-mg tumors were also most active against micrometastatic disease (eg, adriamycin). The converse was also true; agents inactive or marginally active against 40–1000-mg tumors were at best marginally active against micrometastatic disease (eg, BCNU). Tumors > 20 mg were not curable by chemotherapy alone, although adriamycin treatment caused complete regressions of 100–400-mg tumors in > 80% of the mice. Surgical removal of 300–1000-mg tumors plus therapy with adriamycin resulted in 40%–72% cures as compared to 0–26% cures with surgery only. Data resulting from treatment with other agents, singly and in combination, are presented.

[Cancer Treat Rep 62:1471–1488, 1978]

In order to obtain a model for breast cancer in women that could be used for specific drug therapy recommendations, as well as for the study of general principles of adjuvant drug therapy, > 50 C3H spontaneous mammary adenocarcinomas were transplanted and examined for metastatic behavior and also for sensitivity to drugs reported to be active against breast tumors in women. None were found to possess both high metastatic rates and drug responses similar to breast tumors in women. The main deficiency of the tumors studied was variable or low metastatic rates. This problem was compounded when it was noted that the metastatic rate of a tumor did not increase with successive transplant generations. Indeed, the opposite appeared to be the rule. Tumors often lost metastatic abilities if repeatedly transplanted sc from tumors also growing subcutaneously.[5] With this realization, it became clear that the quickest way to obtain and maintain a suitable tumor model was to transplant only metastatic foci. The use of this method for

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[3] Southern Research Institute, Birmingham, Ala.

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**Reprint requests to:* Dr. Thomas H. Corbett, Southern Research Institute, 2000 9th Ave S, Birmingham, Ala 35205.

[5] Corbett TH, Griswold DP, Jr, Roberts BJ, et al. Unpublished observations.

increasing the metastatic rate of a tumor to a specific organ system has precedence in the work of Fidler (1).

MATERIALS AND METHODS

Isolation of 16/C Mammary Adenocarcinoma

The C3H/He strain of mouse has a high incidence of mammary tumors in retired breeders (>9 months old). Many of these tumors are known to be virus-associated (2). Retired breeders (C3H females) were obtained through the National Cancer Institute, Bethesda, Md. Each spontaneous C3H mammary tumor that arose in retired breeders was implanted sc by trocar fragment (~30 mg each) into two 7–10-week-old C3H mice. After the tumors reached 2–3 g in size, the lungs of each mouse were removed and placed into a 1-ml glass syringe (with a Luer-lok). The lung tissue was then injected sc by the technique of Karrer et al (3) through an 18-gauge needle into two 7–10-week-old C3H mice. If the original tumors were highly metastatic, sufficient tumor cells would be present in the transplanted lung tissue to give rise to new tumor growth. 16/C mammary adenocarcinoma was isolated by this technique in 1974.

Maintenance of Metastatic Potential

Most metastatic tumors of mice that we have studied (eg, breast, colon, and melanoma) lose the ability to metastasize at a consistently high rate if maintained exclusively by sc transplantation, with Lewis lung being one of the few exceptions. For this reason, it was often necessary to isolate and transplant metastatic foci in order to maintain this biologic characteristic.

Subcutaneously growing tumors (arising from sc lung tissue implants) were allowed to reach 2–6 g so that the animals were within 7–14 days of death. The animals were killed and the lungs were removed and placed in a chilled Earle's solution. The lung tissue was diced into 20–40-mg fragments and implanted sc with a 12- or 13-gauge trocar into recipient mice. 16/C tumor was maintained by this procedure exclusively.

Determination of Antitumor Activity

Chemotherapy was started within 6 days after tumor implantation (early-stage tumor treatment), or alternatively, chemotherapy was delayed until the tumors became relatively large (~200 mg, advanced-stage tumor treatment). Ten normal mice (with no tumors) were matched to each tumor-bearing treatment group and were injected with the same agents at the same dosages, schedules, days of administration, and drug route as the tumor-bearing group. In this way, drug-induced toxicity (lethality and weight loss) could be clearly separated from the effects of the tumors.

The tumor (16/C) was maintained in serial passage in C3H mice, the host of origin, and transplanted into $B6C3F_1$ mice (C57BL/6 × C3H) for chemotherapy experiments. The animals necessary to begin an experiment were randomized. The nontumor toxicity controls were then removed. The remaining mice were implanted with the tumors (arising from metastatic lung foci) and were again randomized before distribution to the various treatment groups. Tumors were measured with a caliper twice weekly until the death of the animal. Tumor weights were calculated from two-dimensional measurements:

$$\text{Tumor weight (mg)} = \frac{(a \times b^2)}{2}$$

where *a* is the length (mm) and *b* is the width (mm). Mice were weighed at 7-day intervals, beginning the first day of drug treatment or the day prior to the first day of drug treatment; they were checked daily for deaths.

In addition to cure, the following quantitative endpoints were used to assess antitumor effectiveness:

(*a*) Percent increase in lifespan (% ILS) = 100 × (the median day of death [MDD] of the treated-group mice, minus the MDD of the control mice), divided by the MDD of the control mice. Tumor-free survivors were excluded from these calculations.

(*b*) Tumor-growth delay, treated minus control (T–C in days) = the time required for the treatment group tumors (median of ten) to reach 750 mg (or 1250 mg in advanced-stage tumor treatment), minus the time required for the control group tumors (median of ten) to grow to the same size. Tumor-free survivors were excluded from these calculations. The T–C value was the single most important criterion of antitumor effectiveness, and it is essentially the same as the duration of remission value used clinically.

(*c*) Regressions of large tumors: partial regression = > 50% reduction in tumor size; complete regression = regression to below the limit of palpation. Deaths of animals bearing tumors smaller in size than at the first treatment were assumed to be caused by drug toxicity. Regressions that took place in such animals were excluded from the response data.

Surgery-Chemotherapy Adjuvant Treatment

The number of $B6C3F_1$ mice necessary to begin an experiment were randomized. The nontumor toxicity control mice were then removed. These mice were eventually matched to each tumor-bearing and surgery-treatment group and injected with the same agents as the tumor-bearing group. After the removal of the toxicity controls, the remaining mice were implanted with 20–40-mg tumor fragments by trocar into the axillary region. This was considered Day 0 of the experiment. On the day of surgery, animals with tumors of the desired range (300–1000 mg) were selected from the implant pool. The surgery animals were anesthetized with pentobarbital (60 mg/kg) and the tumors were removed with a cutting cautery.[6] Every effort was made to effect 100% removal of the primary site tumor. The surgery animals were placed in a common pool immediately after surgery. They were kept warm until recovery from the pentobarbital was complete and then were unselectively distributed between the surgery-only and the surgery plus chemotherapy groups. The surgery mice were housed three per cage in order to reduce cannibalism. Wound clips were not removed until 14 days after surgery because of retarded wound healing caused by chemotherapy.

In those experiments in which chemotherapy was administered prior to surgery, two surgery-only controls were used (surgery done on 2 different days). The following randomizations were used. On the first surgery day, all animals with 300–1000-mg tumors were pooled. From this pool, mice were distributed to the first surgery-only group, the presurgical chemotherapy groups, and the chemotherapy-only groups. The remainder of the pool was held until the second surgery day at which time the surgery and surgery plus postsurgical chemotherapy groups were handled as previously described for surgical-chemotherapy adjuvant experiments. Tumors were measured with a caliper twice weekly until the death of the animal. All mice were necropsied.

RESULTS

The biologic characteristics of 16/C mammary adenocarcinoma are summarized in table 1. Subcutaneously growing 16/C metastasized primarily to the lungs. In addition, this tumor often metastasized to the axillary lymph nodes, and occasionally to the inguinal nodes. Axillary lymph nodes removed from mice bearing 1–2-g tumors gave rise to new tumor growth (six of 15) when implanted into other C3H mice. Metastatic masses were also detected histologically in axillary lymph nodes of animals bearing far-advanced (4–7-g) subcutaneously growing tumors.

TABLE 1.—Biologic characteristics of 16/C mammary adenocarcinoma

Origin of tumor	Spontaneous (virus associated), 1974
Host of origin	C3H/He
Host used for chemotherapy experiments	$B6C3F_1$ (C57BL/6 female × C3H male)
Histologic description	Grade III, adenocarcinoma
Current transplant generation (Feb 1978)	31
Time required for tumors to grow to 500 mg from a trocar implant*	10–15 days
Median day of death from a trocar implant*	32–46 days
Take rate	
Sc trocar fragment†	100%
Iv counted cells‡	
10^6/mouse	100%
10^5/mouse	80%
Metastasis (from 300- to 1000-mg subcutaneous tumor)	> 75% to lungs > 30% to axillary lymph nodes

*From recent transplant generations.

†Only 1 no-take has been observed in > 10,000 $B6C3F_1$ mice implanted with 16/C trocar fragments.

‡Total cell count: data apply to recent transplant generations.

16/C mammary adenocarcinoma underwent a decrease in tumor volume doubling time (Td) with successive serial transplantation. The overall Td value in early transplants approached 6 days, while tumors from recent transplants (25–31) had Td values of 2.0–3.0 days. Photographs of histologic sections are shown in figure 1. The transplanted tumor is characteristically a moderately differentiated cystadenocarcinoma composed of polygonal cells that form irregularly shaped acini.

Table 2 lists chemotherapy results of early-stage tumor treatment (injections started prior to Day 7 after implant) and advanced-stage tumor treatment (injections started > 9 days after implant). Tumor mass plots of advanced-stage tumor treatments are shown in figure 2. A comparison of the drug responses of 16/C and two other transplantable mouse mammary adenocarcinomas (13/C and 04/A) with human breast tumors (4–6) is summarized in table 3. 04/A adenocarcinoma had a high metastatic rate in early transplant generations but became nearly nonmetastatic after continuous sc to sc transplant.

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[6] Birtcher blendtome electro-surgical unit Model 755.

TABLE 2.—Single agents tested against 16/C mammary adenocarcinoma in B6C3F$_1$ mice*

Transplant generation	Treatment				Toxicity deaths		Median lifespan (days) of controls	% ILS of treated group	Duration of remission: T-C value (days)	Regressions		Tumor-free survivors		Activity rating†
	NSC No.	Agent	MTD (mg/kg/dose)	Drug route and schedule	Tumor mice (No. of deaths/total No. of mice)	Normal mice (No. of deaths/total No. of mice)				Partial (No. of regressions/total No. of mice)	Complete (No. of regressions/total No. of mice)	Control (No. of survivors/total No. of mice)	Treated (No. of survivors/total No. of mice)	
04	123127	Adriamycin	7.8	Iv, Days 5, 12, 19, 26	0/10	0/10	80	19	25.1	—	—	0/10	0/10	+++
25			7.4	Iv, Days 14, 21	1/10	0/10	33.5	91	22.1	9/10	9/10	0/10	0/10	++++
13	739	Aminopterin	1.2	Ip, qd Days 3–7	0/10	0/10	28.5	0	2.2	—	—	0/10	0/10	-
12	141537	Anguidine	2.8	Oral, qd Days 3–6	1/6	—	40.5	22	6.0	—	—	0/20	0/6	+
22			8.0	Iv, Days 13, 20, 27	0/10	0/10	50.0	0	3.0	0/10	0/10	0/10	0/10	-
24	196473	Anthracenedione	18.0	Iv, Days 11, 15, 19, 23	2/10	0/5	35.5	20	7.7	2/10	1/10	0/10	0/10	++
26	287513	Anthracenedione acetate	13.3	Iv, Days 3, 5, 7, 9, 11, 13, 15	0/10	—	48.5	0	10.3	—	—	0/10	0/10	++
28			30.0	Iv, Days 10, 12, 14, 16, 18, 20, 22	1/10	0/10	26	52	16.6	7/10	2/10	0/10	0/10	++
20	102816	5-Azacytidine	3.9	Ip, qd Days 3–7	0/10	0/10	(23.5)‡	(117)‡	3.3	—	—	0/10	0/10	-
22	182986	Aziridinylbenzoquinone	6.9	Iv, Days 3, 7, 11	0/10	—	38.5	2	6.7	—	—	0/10	0/10	+
11	409962	BCNU	15.0	Iv, Days 13, 20, 27	0/10	1/10	32	15	4.0	0/10	0/10	0/10	0/10	-
28			14.5	Iv, Days 3, 7, 11	0/10	0/9	21.5	51	2.7	—	—	0/10	0/10	-
16	125066	Bleomycin	26.0	Iv, Days 3, 10, 17	0/10	—	40	30	5.8	—	—	0/10	0/10	-
20	165563	Bruceantin	1.0	Ip, qd Days 3–7	1/10	1/10	(23.5)‡	(131)‡	2.1	—	—	0/10	0/10	-
24	79037	CCNU	25.0	Oral, Days 3, 10	1/10	—	22	9	2.2	—	—	0/10	0/10	-
13	178248	Chlorozotocin	7.3	Iv, Days 3, 10, 17, 24	0/10	—	29	37	8.3	—	—	0/10	0/10	-
15	119875	*cis*-Dichlorodiammineplatinum(II)	7.5	Iv, Days 3, 10, 17, 24	0/10	—	36.5	10	18.4	—	—	0/10	0/10	++
15			5.2	Iv, Days 11, 18, 25	0/10	0/10	52.0	1	1.2	0/10	0/10	0/10	0/10	-
22	1026	Cycloleucine	64.0	Iv, Days 3, 7, 11, 15	0/10	—	38.5	-29	2.5	—	—	0/10	0/10	-
04	26271	Cyclophosphamide	80.0	Ip, Days 5, 12, 19, 26	0/10	0/10	79.5	10	27	—	—	0/10	0/10	+++
10			91.0	Iv, Days 13, 20, 27	0/10	2/10	34.0	60	13.3	0/10	0/10	0/10	0/10	++
25	82151	Daunorubicin	13.0	Iv, Days 14, 21	0/9	0/5	40.5	55	21.1	9/9	8/9	0/10	0/9	+++

24	19494	DDMP	3.0	Ip, qd Days 2–6	1/10	—	45.0	2	5.7	—	—	0/10	0/10	+
21	261726	3-Deazaguanine	47.0	Ip, qd Days 1–9	0/10	—	42.5	31	9.5	—	—	0/10	0/10	++
13	104800	Dibromodulcitol	300.0	Ip, Day 2	0/10	0/10	28.0	27	13.8	—	—	0/10	0/10	++
06			430.0	Oral, Days 22, 29, 36	0/10	0/10	63.0	14	20.2	6/10	4/10	0/10	0/10	+++
21	118994	Diglycoaldehyde	290.0	Ip, Days 3, 4	1/10	0/10	26.5	41	2.6	—	—	0/10	0/10	–
27	7365	DON	26.0	Iv, Days 3, 7, 11, 15	2/10	—	24.5	16	10.5	—	—	0/10	0/10	+
16	45388	DTIC	320.0	Ip, Days 3, 10, 17	1/10	—	40.0	7	4.0	—	—	0/10	0/10	–
26	71795	Ellipticine	46.0	Sc, Days 11, 15, 19, 23	0/10	1/10	33	28	2.6	0/10	0/10	0/10	0/10	–
24			67.0	Sc, Days 3, 7, 10	0/10	—	39.0	41	15.1	—	—	0/10	0/10	+++
08	148958	Ftorafur	170.0	Sc, qd Days 3–7, 14–18	1/10	1/10	27	66	10.4	—	—	0/10	0/10	+
16			480.0	Ip, Days 3, 10, 17	0/10	—	40	30	6.6	—	—	0/10	0/10	–
08	19893	5-Fluorouracil	22.0	Sc, qd Days 3–7, 14–18	2/10	1/10	27	140	19.5	—	—	0/10	0/10	+++
17			50.0	Iv, Days 8, 15, 22, 29	0/24	—	29	155	24.9	—	—	0/25	0/24	+++
22			56.0	Iv, Days 11, 15, 19	1/9	0/10	30	70	13.4	7/9	5/9	0/10	0/9	+++
33			23.0	Ip, qd Days 3–10	0/10	—	28	1	6.1	—	—	0/10	0/10	+
16	13875	Hexamethylmelamine	300.0	Ip, Days 3, 10, 17	1/10	—	40	2	5.5	—	—	0/10	0/10	–
20	141633	Homoharringtonine	3.1	Iv, Days 3, 7, 11	0/10	1/10	23.5	76	6.3	—	—	0/10	0/10	+
21	246112	Hydrazinecarbothioamide	64.0	Ip, Days 3, 4	1/10	0/10	26.5	66	1.8	—	—	0/10	0/10	–
16	129943	ICRF-159	260.0	Ip, Days 3, 10, 17	1/10	—	40	20	3.9	—	—	0/10	0/10	–
21	529469	L-Alanosine	71.0	Ip, qd Days 3–7	1/10	0/10	26.5	64	6.3	—	—	0/10	0/10	+
12	208642	Lymphosarcin	13.0	Iv, Days 3, 10, 17	0/6	—	40.5	30	21.3	—	—	0/20	0/6	++→+++
20			4.5	Oral, qd Days 3–7, 13–17, 23–27	1/10	0/9	23.5	65	2.0	—	—	0/10	0/10	–
14	153858	Maytansine	0.8	Iv, Days 11, 18, 25	1/10	0/10	31.5	71	15.5	3/10	1/10	0/10	0/10	++
11	8806	Melphalan	12.0	Ip, Day 2	1/10	0/10	37	21	9.0	—	—	0/10	0/10	++
04			8.6	Ip, Days 5, 12, 19, 26	0/10	2/10	80	20	33.9	—	—	0/10	0/10	+++
07			11.5	Ip, Days 15, 22	0/10	1/10	33.5	43	18.7	8/10	8/10	0/10	0/10	++
26			5.0	Iv, Days 11, 15, 19, 23	0/10	0/10	33.0	27	10.0	0/10	0/10	0/10	0/10	+
04	740	Methotrexate	56.0	Iv, Days 5, 12, 19, 26	0/10	0/10	79.5	7	0	—	—	0/10	0/10	–
11	26980	Mitomycin C	4.0	Iv, Day 2	0/10	0/10	34.0	20	9.9	—	—	0/20	0/10	++
08			3.0	Iv, Days 10, 17, 24	0/10	0/10	39.5	39	18.9	4/10	1/10	0/10	0/10	++

TABLE 2.—Single agents tested against 16/C mammary adenocarcinoma in B6C3F$_1$ mice*—Continued

	Treatment				Toxicity deaths					Regressions		Tumor-free survivors		
Transplant generation	NSC No.	Agent	MTD (mg/kg/dose)	Drug route and schedule	Tumor mice (No. of deaths/total No. of mice)	Normal mice (No. of deaths/total No. of mice)	Median lifespan (days) of controls	% ILS of treated group	Duration of remission: T-C value (days)	Partial (No. of regressions/total No. of mice)	Complete (No. of regressions/total No. of mice)	Control (No. of survivors/total No. of mice)	Treated (No. of survivors/total No. of mice)	Activity rating†
13	157365	Neocarzinostatin	0.9	Iv, Days 3, 10, 17	2/10	—	57	14	18.9	—	—	0/10	0/10	++
24	224131	PALA	190.0	Ip, qd Days 3–11	0/10	—	18	16	5.7	—	—	0/10	0/10	+
15	135962	PalmO-ara-C	88.0	Ip, Days 11, 18, 25, 32	1/10	2/10	36.5	83	27.1	2/10	2/10	0/10	0/10	+++
24	95466	PCNU	13.0	Oral, Days 3, 10	1/10	—	22	18	3.3	—	—	0/10	0/10	-
23	118742	Pentamethylmelamine-HCl	230.0	Ip, Days 3, 10, 17	0/10	—	40.0	-19	3.9	—	—	0/10	0/10	-
15	135758	Piperazinedione	8.0	Iv, Days 3, 10, 17, 24	0/10	—	36.5	60	18.4	—	—	0/10	0/10	++
16			7.2	Iv, Days 12, 19	0/10	0/10	23.5	117	5.8	0/10	0/10	0/10	0/10	+
04	10023	Prednisone	180.0	Sc, Days 5, 12, 19, 26	0/10	3/10	80	23	27.1	—	—	0/10	0/10	++
31			140.0	Sc, Days 10, 14, 18	0/10	1/8	24.0	54	-0.1	0/10	0/10	0/10	0/10	-
25	164011	Rubidazone	14.0	Iv, Days 14, 21	0/10	0/5	40.5	23	13.9	6/10	3/10	0/10	0/10	++
15	85998	Streptozotocin	80.0	Iv, Days 3, 7, 11, 15	0/10	—	40.0	-29	1.0	—	—	0/10	0/10	-
24	125973	Taxol	62.0	Ip, Days 3, 7, 11, 15	0/10	—	40.0	-30	1.6	—	—	0/10	0/10	-
22	71261	TGdR	28.0	Iv, Days 3, 7, 11, 15	1/10	—	38.5	-8	7.4	—	—	0/10	0/10	+
13	4728	Thiadiazole	105.0	Ip, Days 3–8	0/6	—	57.0	0	4.3	—	—	0/10	0/6	-
15	752	6-Thioguanine	17.3	Iv, Days 3, 10, 17, 24	1/10	—	36.5	16	5.0	—	—	0/10	0/10	-
24	283162	Trimethyltrimethylolmelamine	200.0	Ip, Days 3, 7	0/10	—	40.0	21	8.7	—	—	0/10	0/10	++
15	49842	Vinblastine	7.5	Iv, Days 3, 10, 17, 24	0/10	—	36.5	69	21.9	—	—	0/10	0/10	+++
08			4.7	Iv, Days 10, 17, 24	0/10	0/10	39.5	16	7.2	1/10	1/10	0/10	0/10	-
04	67574	Vincristine	2.2	Iv, Days 5, 12, 19, 26	0/10	2/10	79.5	32	21.1	—	—	0/10	0/10	+++
06			2.3	Iv, Days 25, 32, 39, 46	2/10	1/10	63.5	33	28.6	4/10	3/10	0/10	0/10	+++

13	245467	Vindesine	3.2	Iv, Days 3, 10, 17	0/9	—	57.0	1	10.3	—	—	0/9	0/10	+
12	122819	VM-26	12.0	Iv, Days 3, 10, 17, 24	0/6	—	40.5	50	16.9	—	—	0/10	0/6	++
15			10.0	Iv, Days 11, 18	2/10	—	32.0	0	10.7	3/10	1/10	0/10	0/10	+
13	141540	VP-16-213	24.5	Iv, Days 3, 10, 17, 24	1/10	—	29	25	10.2	—	—	0/10	0/10	+
20			21.0	Iv, Days 15, 22, 29	0/10	0/10	50	18	13.1	1/10	1/10	0/10	0/10	+

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*Treatment schedule: treatment initiated prior to 7 days after implant was considered to be early-stage treatment. The antitumor activity of an agent was usually superior against early-stage disease.

†Activity ratings dependent on T–C values were occasionally modified in light of ILSs, tumor regressions, and transplant generation. (T–C values tended to be higher in early transplant generations.) No agent was ranked + + + + unless an ILS of > 45% was obtained in addition to the required T–C value.

Activity rating	T–C value (duration of treatment ≤ 12 days)	T–C value (duration of treatment > 12 days)
Highly active ++++	> 16	> 25
+++	12–16	19–25
++	8–11	14–18
+	5–7	9–13
Inactive –	< 5	< 9

‡Control mice died in a shorter period of time than usual. % ILS value should be discounted.

13/C adenocarcinoma was isolated by the selection technique described for 16/C. The suitability of the 13/C tumor for surgical-chemotherapy adjuvant experiments is currently under evaluation.

Figure 3 is a cumulative mortality plot of $B6C3F_1$ mice after surgical removal of 300–1000-mg subcutaneous 16/C mammary tumors. Because cures were obtained in these experiments, it can be assumed that the last few animals that died had only a very small number (approaching one) of clonigenic cells present immediately after surgery. This fact, used in conjunction with the number of doublings required for one cell to reach the lethal number (3×10^8 cells in the lungs, which equal 27 doublings), allows a crude calculation of the overall Td for this tumor.

$$\frac{50}{27} \rightarrow \frac{84}{27} = (2 \rightarrow 3 \text{ days})$$

The wide range of deaths (fig 3) indicates a large difference among the mice with respect to the likely tumor cell burden remaining after surgery. Some of the mice had no clonigenic tumor cells (cures), while others had $\leq 10^7$ cells.

Twenty-four consecutive surgery-chemotherapy adjuvant experiments were carried out with mice bearing 300–1000-mg subcutaneous tumors of 16/C mammary adenocarcinoma. In order to indicate the rate of metastasis of tumors in this size range, the causes of deaths in the surgery-only groups were tabulated: 12 deaths occurred from primary site regrowths (or axillary lymph node metastasis) without gross evidence of distant metastasis; 154 deaths occurred with primary site regrowths (or axillary lymph node metastasis) plus distant metastasis (usually lung); 189 deaths occurred from distant metastasis without primary site regrowths (or lymph node metastasis); 18 deaths occurred from nontumor causes or no necropsy could be performed because of cannibalism; and 60 mice were cured. Distant metastasis occurred in ≥ 80% of the mice in 17 of 24 experiments, between 70% and 80% in five of 24 experiments, and between 60% and 70% in two of 24 experiments.

Six examples of surgery-chemotherapy adjuvant experiments are shown in table 4 and two of these are shown in figures 4 and 5. These examples were selected from different transplant generations. The interval between implantation of the tumor and the surgical removal of the tumor decreased progressively with successive transplant generations. This was because the tumors reached a 300–1000-mg size more rapidly with successive transplants. Surgery plus chemotherapy (with active agents) increased (*a*) the median tumor-free interval, (*b*) the median survival time, and (*c*) the number of mice cured over that of surgery alone. Experiments were carried out in an attempt to determine the most effective time to begin chemotherapy in relation to surgery. Chemotherapy administered 2 or 3 days prior to surgery clearly produced more cures than when administered 3 days after surgery (table 4, experiments 1513 M1, O1, and P1; fig 5). In other experiments (data not shown) with this tumor and other metastatic tumors, chemotherapy that was started the day of, the day before, or the day after surgery (with agents such as adriamycin, methyl-CCNU, or BCNU + 5-fluorouracil) was not superior to chemotherapy (at the maximum tolerated dosage [MTD]) that was started 3 or 4 days after surgery. These results are probably related to the finding that the MTD of chemotherapy given within 1 day of surgery was most often only 40%–50% of the MTD given 3–4 days after surgery. Host debilitation induced by the tumor in conjunction with surgical trauma and anesthetic toxicity appeared to affect drug lethality. The MTD of chemotherapy was approximately the same 2 or 3 days prior to surgery compared to 3 days after surgery (table 4, experiments 1513 M1, O1, and P1).

There has often been a misunderstanding among those not directly involved in the treatment of experimental tumors as to the relationship between the stage of the tumor growth and the apparent effectiveness of the antitumor agent. It is generally recognized that antitumor activity of a given active drug will increase as the tumor burden is reduced. If treatment was started soon after tumor implantation (eg, Day 3 after implant) while the tumors were only 20–40 mg in size, antitumor activity was generally greater (as measured by T–C values [tumor-growth delay]) than if treatment was delayed until the tumors reached the 100–300-mg size (Day 10 or later after implant) [table 2; eg, *cis*-dichlorodiammineplatinum(II), cyclophosphamide, piperazinedione, prednisone, and vinblastine]. Nonetheless, if the agent was highly active against the 20–40-mg tumor (+++ → ++++ activity rating), it was still likely to retain significant activity against the 100–300-mg size tumor masses (table 2; eg, adriamycin, cyclophosphamide, 5-fluorouracil, melphalan, and vincristine). If, however, treatment was delayed still further until the tumors were ≥ 300–1000 mg (2%–5% of the animal's body weight), even very effective agents or combinations were unable to halt the tumor growth or add significantly to the animal's lifespan (table 4; eg, adriamycin, adriamycin + cyclophosphamide, and adriamycin + dianhydrogalactitol). However, if the tumor burden was reduced by surgery to $< 10^6$ cells, those remaining

TABLE 3.—Comparison of antitumor activity against 3 transplantable mouse mammary adenocarcinomas and human mammary tumors*

Agent	Line 04/A	Line 13/C	Line 16/C	Human mammary tumors (ref No.)
Adriamycin	+→++++	+++→++++	+++→++++	++ (4,6)
Aminopterin			−	Unknown
Anguidine		−	−→+	Unknown
Anthracenedione (HCl or acetate)		+++	++	Unknown
5-Azacytidine		−→+	−	− (4)
Aziridinylbenzoquinone		+	+	Unknown
BCNU	−	−	−	−→+ (4)
Bleomycin		−	−	− (4)
Bruceantin			−	Unknown
CCNU			−	Unknown
Chlorozotocin			−	Unknown
cis-Dichlorodiammine-platinum(II)			−→+++	−→± (5)
Cycloleucine		−	−	Unknown
Cyclophosphamide	++→++++	++→++++	++→+++	++ (4)
Daunorubicin			+++	Unknown
DDMP		+	+	Unknown
3-Deazaguanine		++	++	Unknown
Dibromodulcitol	++++	+	++→+++	+ (4)
Diglycoaldehyde		−	−	Unknown
DON			+	Unknown
DTIC			−	− (4)
Ellipticine		+++	−→+++	Unknown
Ftorafur			−→+	Unknown
5-Fluorouracil	+→++++	+→+++	+→+++	++ (4,6)
Hexamethylmelamine	−		−	+ (4)
Homoharringtonine			+	Unknown
Hydrazinecarbothioamide			−	Unknown
ICRF-159			−	Unknown
L-Alanosine			+	Unknown
Lymphosarcin		++	−→+++	Unknown
Maytansine		++	++	Unknown
Melphalan	+→++++	+++	+→+++	++ (4)
Methotrexate	−	−	−	++ (4,6)
Mitomycin C	+++	+++	++	++ (4)
Neocarzinostatin		++	++	Unknown

TABLE 3.—Comparison of antitumor activity against 3 transplantable mouse mammary adenocarcinomas and human mammary tumors*—Continued

Agent	Line 04/A	Line 13/C	Line 16/C	Human mammary tumors (ref No.)
PALA	++	–→+++	+	Unknown
PalmO-ara-C	+++	+	+→+++	Unknown
PCNU			–	Unknown
Pentamethylmelamine-HCl			–	Unknown
Piperazinedione		+++	+→++	Unknown
Prednisone	+→++++	+	–→++	Unknown
Pyrazofurin			+	Unknown
Rubidazone			+++	Unknown
Streptozotocin			–	– (4)
Taxol			–	Unknown
β-TGdR			+	Unknown
Thiadiazole			–	Unknown
6-Thioguanine			–	Unknown
Trimethyl-trimethyl-ol-melamine		–	++	Unknown
Vinblastine	+++	–→+++	–→+++	+ + (4)
Vincristine	–→++	+→+++	+++	++ (4,6)
Vindesine		++++	+	Unknown
VM-26		+	+→++	Unknown
VP-16-213		+	+→+++	Unknown

*See table 2 for definitions of activity ratings.

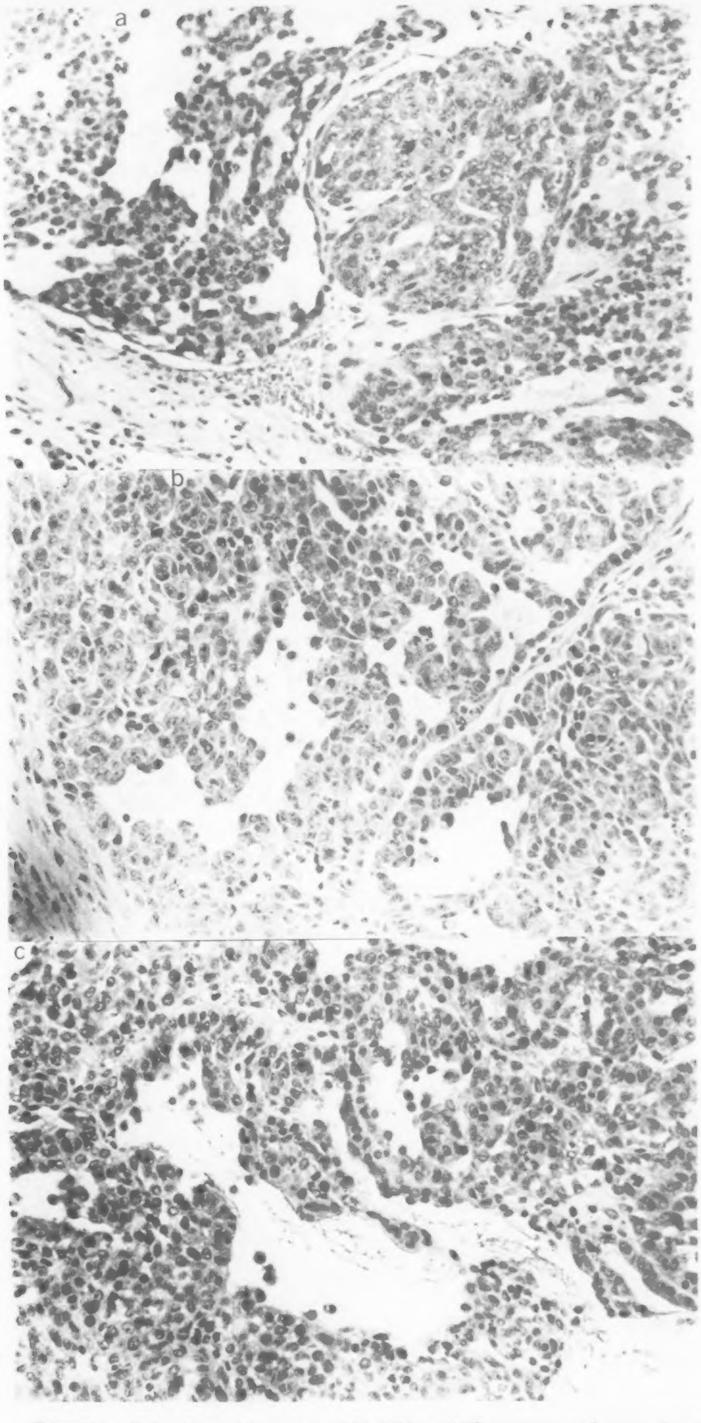

FIGURE 1.—Subcutaneously growing 500–2000-mg 16/C mammary adenocarcinoma tumors (H & E stain, × 250). Transplant generations Nos. 21 and 22.

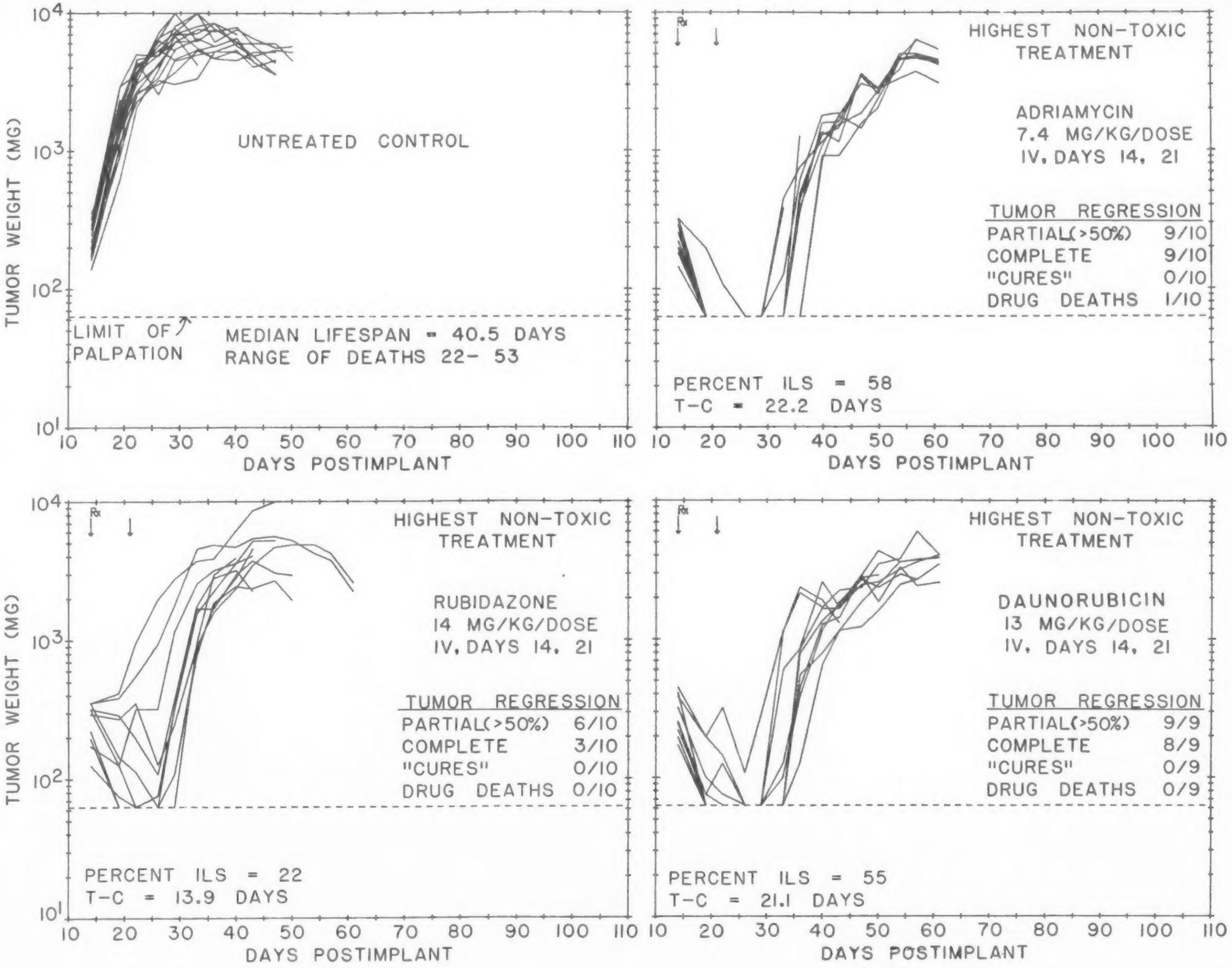

FIGURE 2.—B6C3F$_1$ mice bearing 100–400-mg 16/C mammary tumors were treated with adriamycin, rubidazone, and daunorubicin at a range of dosages. The results of the highest nontoxic treatment (≤ LD10) were compared in tumor mass plots. Transplant generation No. 25, experiment 1552 W1.

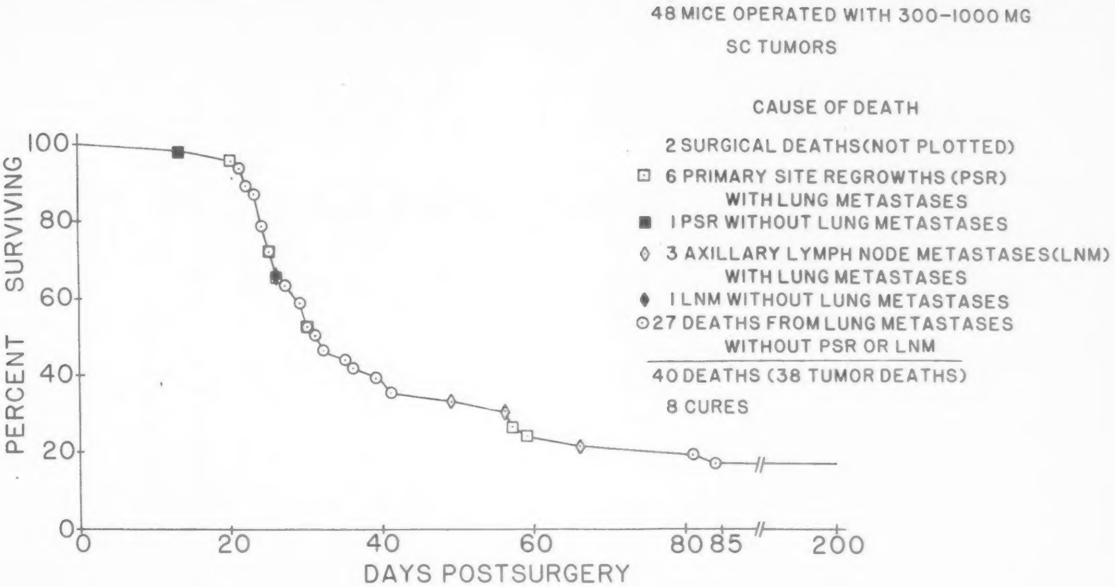

FIGURE 3.—Cumulative mortality of B6C3F$_1$ mice after surgical removal of 300–1000-mg subcutaneous 16/C mammary tumors is plotted (pooled results of 2 experiments). Transplant generations Nos. 20 and 22.

cells were at least as responsive and usually more responsive than small tumor masses in the 20–40-mg range (see % ILS in tables 2 and 4; eg, adriamycin). If the agent, however, was inactive or marginally active against 20–40-mg tumor masses (early-stage disease), it was invariably inactive against larger tumor masses (table 2; eg, anguidine and BCNU). Most important, however, was the fact that such a marginally active or inactive agent did not display any significant activity against micrometastatic disease (7). An example of this also can be seen in the data presented here; BCNU (as well as other nitrosoureas—PCNU, CCNU, and chlorozotocin) was inactive (or at best only marginally active against 16/C mammary adenocarcinoma [table 2]). BCNU was also essentially inactive against micrometastatic disease (table 4, experiment 1513 D1). In addition, after the tumor burden was reduced still further by adriamycin (administered at 7.5 mg/kg/dose, 3, 10, and 17 days after surgery), BCNU still did not display activity (table 4).

DISCUSSION

16/C mammary adenocarcinoma fulfilled the two requirements of a suitable surgery-chemotherapy model; first, it possessed drug response characteristics similar to many mammary tumors in women, and second, it metastasized in 70%–100% of the mice bearing 300–1000-mg subcutaneous tumors (343 distant metastases in 433 mice). Metastasis occurred not only to the lungs from an sc implant site but also occurred frequently in the axillary lymph nodes. Metastases were also occasionally seen in the kidneys, ovaries, and mesentery. The surgery-chemotherapy adjuvant results with 16/C mammary adenocarcinoma were consistent with previously reported findings with colon carcinomas (7). That is, if an agent or combination was effective (+++ → ++++ activity rating) against small (20–40 mg) or moderately advanced tumor masses (100–300 mg), this treatment was found to be as effective and usually more effective against micrometastatic disease. The converse was also true. An agent marginally active or inactive against 20–40-mg tumors did not become more effective against micrometastatic disease. The time at which chemotherapy was started influenced the curability of 16/C mammary adenocarcinoma. In three separate experiments, if chemotherapy was administered 2 or 3 days prior to the surgery with or without postsurgical chemotherapy, the tumor was cured more frequently than when chemotherapy was started 3 or more days after surgery. Further experiments are in progress to confirm these results. Cyclophosphamide administered 4 days prior to surgical removal of B16 melanoma has been reported to be superior to postsurgical chemotherapy (8).

TABLE 4.—Surgery + chemotherapy adjuvant treatment of 16/C mammary adenocarcinoma

Treatment	Agent and route	MTD (mg/kg/ dose)	Schedule of drug admin-istra-tion*	Day† of tumor re-moval‡	MDD†·§ (range of tumor deaths)	% ILS¶	Deaths from factors other than tumor growth**	Deaths with primary site growth or re-growth without metas-tasis††,‡‡	Deaths with primary site growth or re-growth with metas-tasis††	Deaths from metas-tasis without primary site re-growth	Tumor-free survivors (cures)
Experiment No. 1513 C1 (experiment terminated on Day 250; tumor in 7th transplant generation)§§											
No treatment control	—	—	—	—	43(27–76)	—	0/15	9/15	6/15	—	0/15
Chemotherapy only	Adriamycin, iv Cyclophosphamide, ip	7 110	24 24	—	47(25–87)	9	1/10	5/10	4/10	—	0/10
Chemotherapy only	Adriamycin, iv Cyclophosphamide, ip	4.2 80.0	24,31,38 24,31,38	—	43.5(25–103)	1	1/10	6/10	3/10	—	0/10
Surgery only	—	—	—	24	88(58–198)	102	2/15	2/15	6/15	4/15	1/15
Surgery + chemotherapy	Adriamycin, iv Cyclophosphamide, ip	7 110	27 27	24	109(77–174)	153	0/15	0/15	3/15	7/15	5/15
Surgery + chemotherapy	Adriamycin, iv Cyclophosphamide, ip	4.2 80	27,34,41 27,34,41	24	151(44–226)	237	0/14	0/14	0/14	9/14	5/14
Experiment No. 1513 D1 (experiment terminated on Day 224; tumor in 8th transplant generation)§§											
No treatment control	—	—	—	—	45(31–58)	—	0/15	0/15	15/15	—	0/15
Chemotherapy only	Adriamycin, iv	7.5	20,27,34,41	—	90(59–102)	100	0/10	1/10	9/10	—	0/10
Chemotherapy only	BCNU, iv	32.0	20	—	46.5(40–64)	3	0/10	2/10	8/10	—	0/10
Chemotherapy only	Adriamycin, iv BCNU, iv	7.5 32.0	20,27,34 41	—	78(65–119)	73	0/10	4/10	6/10	—	0/10
Surgery only	—	—	—	20	52.5(42–81)	17	1/15	0/15	9/15	5/15	0/15
Surgery + chemotherapy	Adriamycin, iv	7.5	23,30,37,44	20	119(100–224)	164	0/15	1/15	6/15	8/15	0/15
Surgery + chemotherapy	BCNU, iv	43.0	23	20	54(43–72)	20	1/15	0/15	11/15	3/15	0/15
Surgery + chemotherapy	Adriamycin, iv BCNU, iv	7.5 24.0	23,30,37 44	20	88(72–120)	95	1/15	1/15	5/15	8/15	0/15
Experiment No. 1513 G1 (experiment terminated on Day 261; tumor in 10th transplant generation)§§											
No treatment control	—	—	—	—	48(28–66)	—	0/15	5/15	10/15	—	0/15
Chemotherapy only	Adriamycin, iv	7.2	24,31,38,45	—	60(52–85)	25	0/10	0/10	10/10	—	0/10
Surgery only	—	—	—	21	52(31–191)	8	0/15	1/15	9/15	5/15	0/15
Surgery + chemotherapy	Adriamycin, iv	7.2	24,31,38,45	21	109(72–228)	127	0/15	1/15	4/15	4/15	6/15

Experiment No. 1513 M1 (experiment terminated on Day 260; tumor in 12th transplant generation)											
No treatment control	—	—	—	—	46(32–65)	—	0/15	7/15	8/15	—	0/15
Chemotherapy only	Adriamycin, iv Dianhydrogalactitol, iv	5 4	16,23 16,23	—	49(36–72)	7	0/10	8/10	2/10	—	0/10
Surgery only	—	—	—	14	50(33–131)	9	0/15	1/15	9/15	5/15	0/15
Surgery only	—	—	—	16	55(33–93)	20	1/15	1/15	6/15	5/15	2/15
Surgery + chemotherapy	Adriamycin, iv Dianhydrogalactitol, iv	5 4	19,26 19,26	16	81(53–139)	76	0/15	2/15	6/15	7/15	0/15
Surgery + chemotherapy	Adriamycin, iv Dianhydrogalactitol, iv Adriamycin, iv Dianhydrogalactitol, iv	3.3 2.7 5 4	14 14 21 21	16	127(42–178)	176	3/20	1/20	0/20	9/20	7/20
Experiment No. 1513 O1 (experiment terminated on Day 320; tumor in 22nd transplant generation)											
No treatment control	—	—	—	—	37(24–64)	—	0/15	9/15	6/15	—	0/15
Chemotherapy only	Adriamycin, iv	9	12	—	52.5(38–74)	41	0/10	5/10	5/10	—	0/10
Chemotherapy only	Adriamycin, iv	5.8	12,18	—	40.5(37–81)	9	0/10	7/10	3/10	—	0/10
Surgery only	—	—	—	12	41(33–78)	10	2/18	0/18	3/18	10/18	3/18
Surgery only	—	—	—	15	40(36–63)	8	0/15	0/15	1/15	10/15	4/15
Surgery + chemotherapy	Adriamycin, iv	9	18	15	58(49–132)	56	3/15	0/15	3/15	7/15	2/15
Surgery + chemotherapy	Adriamycin, iv	9	12	15	87.5(67–191)	136	0/18	2/18	2/18	2/18	12/18
Surgery + chemotherapy	Adriamycin, iv	5.8	12,18	15	72.5(49–252)	96	1/19	1/19	2/19	6/19	9/19
Experiment No. 1513 P1 (experiment terminated on Day 229; tumor in 27th transplant generation)											
No treatment control	—	—	—	—	26(17–47)	—	0/15	12/15	3/15	—	0/15
Chemotherapy only	Adriamycin, iv	14.0	12	—	40(32–57)	53	0/10	8/10	2/10	0/10	0/10
Chemotherapy only	Adriamycin, iv	8.0	12	—	37(32–57)	42	0/10	8/10	2/10	—	0/10
Surgery only	—	—	—	12	48(36–67)	85	0/15	1/15	1/15	8/15	5/15
Surgery only	—	—	—	15	47.5(34–67)	83	1/17	0/17	4/17	10/17	2/17
Surgery + chemotherapy	Adriamycin, iv	14.0	12	15	60(41–105)	130	1/22	0/22	1/22	5/22	15/22
Surgery + chemotherapy	Adriamycin, iv	14.0	15	12	47.5(74–138)	82	4/15	1/15	0/15	3/15	7/15
Surgery + chemotherapy	Adriamycin, iv	8.0	12	15	62(39–133)	138	3/22	2/22	0/22	7/22	10/22
Surgery + chemotherapy	Adriamycin, iv	8.0	15	12	60(50–87)	131	3/15	0/15	1/15	8/15	3/15

*Schedule: in days after implant of tumors (1 injection each day listed).

†Day after implant of 20-mg trocar fragments.

‡All tumors were 300–1000 mg at the time of surgical removal.

§Dying only: cures, missing animals, and accidental deaths were excluded from the MDD calculation. In addition, all other nontumor deaths were excluded from the range of death values.

¶% ILS = 100 × (the MDD of the treated group minus the MDD of the control group) divided by the MDD of the control group.

**Deaths due to after-effects of surgery, drug toxicity, infections, or unknown causes. In some cases a necropsy could not be performed as a result of cannibalism.

††> 30% of the tumors listed as "primary site regrowths" were actually axillary lymph node metastasis.

‡‡Without gross evidence of metastasis or metastasis could not be determined due to cannibalism. Evidence of subcutaneous primary site tumor growth came from prior twice weekly tumor measurements.

§§Mice cured in experiments 1513 C1, D1, and G1 were challenged with trocar implants (20–30-mg fragments) of 16/C. In all cases the implants grew and killed the animals.

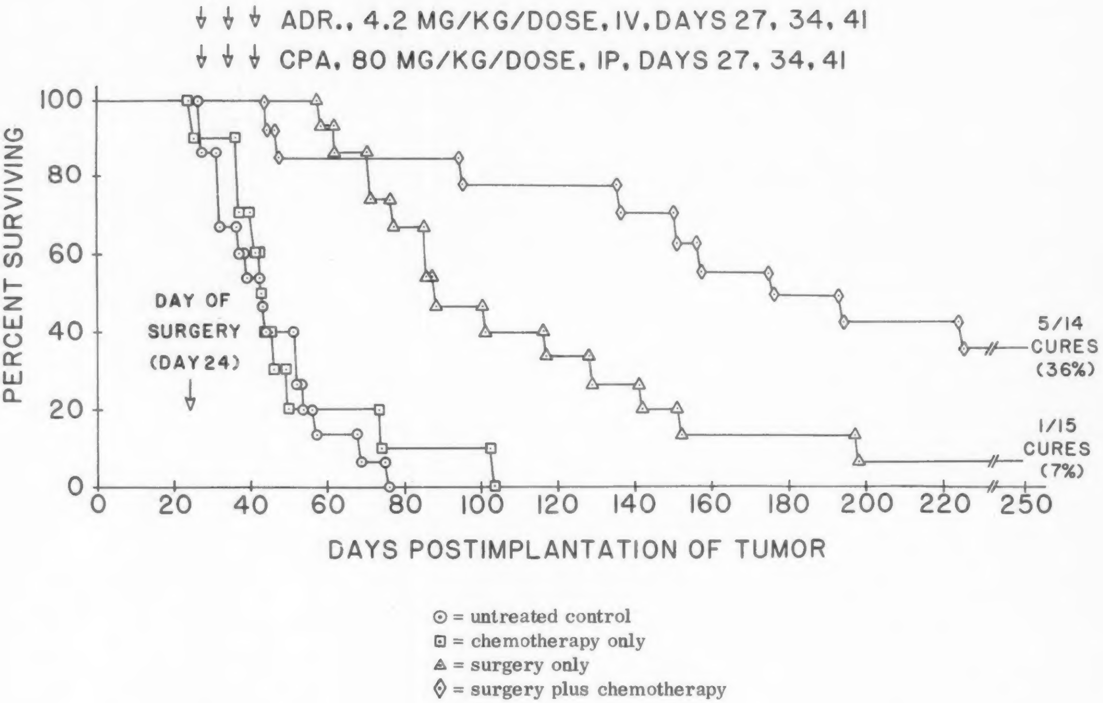

FIGURE 4.—Survival of B6C3F$_1$ mice bearing 300–1000-mg subcutaneous 16/C mammary tumors after surgical excision and treatment with adriamycin + cyclophosphamide. Transplant generation No. 7, experiment 1513 C1.

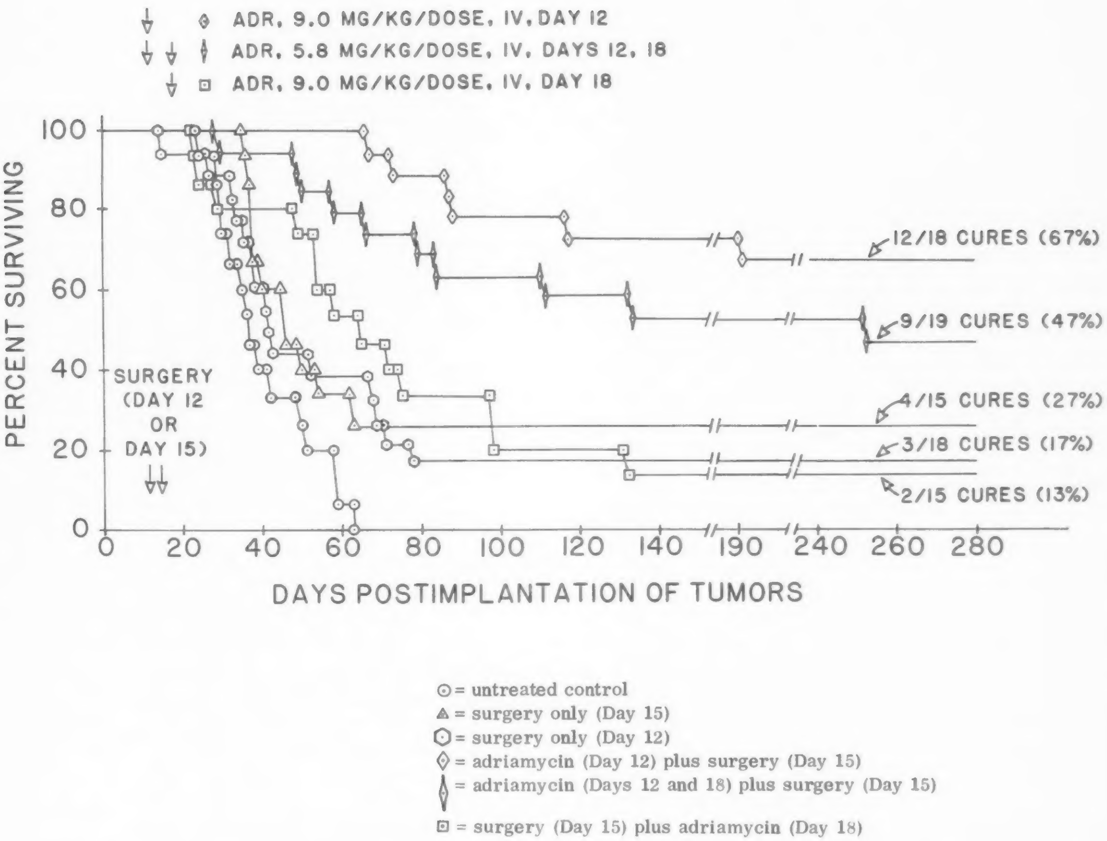

FIGURE 5.—Survival of B6C3F$_1$ mice bearing 300–1000-mg subcutaneous 16/C mammary tumors after surgical excision and treatment with adriamycin either before surgery, after surgery, or before and after surgery. Transplant generation No. 22, experiment 1513 O1.

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Protective Effect of Hyperthermia Against the Cytotoxicity of Actinomycin D on Chinese Hamster Cells [1,2,3]

Sarah S. Donaldson,* Louise F. Gordon, and George M. Hahn [4,5]

SUMMARY

Hyperthermia is being tested as an adjuvant to chemotherapy for clinical use. We elected to study the interaction of heat at 43°C with actinomycin D (AMD) (0.5 μg/ml) in tissue culture using plateau phase Chinese hamster cells. The *simultaneous* administration of 43°C and AMD produces more than additive cytotoxicity if the duration of exposure is $<$ 30 minutes; however, this is quickly reversed with longer exposures with the cells developing resistance to further cytotoxicity of AMD. If heat (43°C) is applied *before* AMD exposure, the cells also are rendered insensitive, with a greater protection observed for longer periods of heating. For example, for cells heated for 2 hours at 43°C and then exposed to AMD (0.5 μg/ml for 2 hours), cytotoxicity to AMD is decreased by a factor of 10. Heat-induced resistance to AMD persists for at least 18 hours before full recovery of AMD effect returns. The application of heat *following* AMD exposure also protects against the cytotoxicity of AMD. Studies using ^{3}H-AMD demonstrate that the resistance does not correlate with reduced membrane permeability to AMD of heated cells. Attention must be given to the timing of hyperthermia when used clinically as adjuvant therapy in patients receiving AMD.

[Cancer Treat Rep 62:1489–1495, 1978]

The renewed interest in whole-body hyperthermia as treatment for advanced malignant disease (1), as well as adjuvant treatment with radiotherapy and chemotherapy (2,3), has expanded the potential for clinical use of combined modality treatment and has been the nidus for clinical trials using hyperthermia (4,5).

A review of experimental observations and discussion of mechanisms of the apparent selective lethal effect of hyperthermia in the range of 41°C–43°C on tumor cells has recently been published (6). Hyperthermia has been shown to enhance the effects of several chemotherapeutic agents, including adriamycin and bleomycin (7). Previous experiments in our laboratories suggest that the timing of hyperthermia and adriamycin may be critical, and that protection against known cytotoxicity of adriamycin may occur in vitro when heat at 43°C and adriamycin are administered simultaneously for extended periods of time or when cells are exposed to heat prior to adriamycin (8).

Although adriamycin and actinomycin D (AMD) differ in molecular structure, both antibiotics bind to DNA by intercalation between base pairs (9,10), inhibit DNA-dependent RNA synthesis (9,11), inhibit repair processes in irradiated mammalian cells (12,13), exhibit the "recall phenomenon" when used in conjunction with radiotherapy (14,15), and have demonstrated antineoplastic activities in a variety of tumors. The present study was undertaken to investigate the interaction of hyperthermia at 43°C with AMD, a well-established and widely utilized

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[4] Department of Radiology, Stanford University Medical Center, Calif.

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**Reprint requests to:* Sarah S. Donaldson, MD, Department of Radiology, Stanford University Medical Center, Stanford, Calif 94305.

antibiotic chemotherapeutic agent with efficacy in pediatric as well as adult neoplasms.

MATERIALS AND METHODS

Cells

Chinese hamster (HA-1) cells were grown in Eagle's minimal essential medium (MEM) supplemented with 15% fetal calf serum, penicillin, and streptomycin. The cultures were kept in a humidified incubator with a mixture of 95% air and CO_2 and routinely checked for mycoplasma. Density-inhibited cultures (16) (plateau phase) were obtained by plating 1–2 × 10^5 cells in 60-mm glass petri dishes. When the cell number reached 2 × 10^6 cells/dish, the medium was changed daily. Under these conditions, the maximum cell density was about 2 × 10^7 cells/dish. Experiments were performed on the second day of plateau phase.

Assay for cell viability was accomplished by harvesting cells with 0.05% trypsin, counting cell numbers on a Coulter counter, and plating appropriate dilutions in Eagle's MEM to yield approximately 200 colonies/60-mm petri dish. After 10 days of incubation at 37°C, colonies were fixed and stained with 0.25% crystal violet and counted; corrections were made for plating efficiency and surviving fractions were determined. Plating efficiencies were 60%–80%.

Drug Treatment

AMD was mixed in preservative-free sterile water, diluted in Eagle's MEM supplemented with 15% fetal calf serum to a concentration of 0.5 μg/ml, protected from light, and kept on ice throughout each experiment. The identical serum lot was used for the entire series of experiments. Previous experiments had shown that the addition of up to 10^5 cells killed by AMD (0.5 μg/ml, 2 hours) did not affect the plating efficiency of cells not exposed to AMD. Cells exposed to higher doses of AMD did have such an effect. Hence, the dose used in experiments was limited to 0.5 μg/ml.

Following AMD exposure, the cells were rinsed three times with phosphate buffered saline and then overlaid with 5 ml of Eagle's MEM with 15% fetal calf serum. Control experiments were performed to demonstrate that following this rinsing procedure, there was no continued drug action by 0.5 μg/ml of AMD bound to the glass petri dishes. Dishes containing no cells were exposed to graded doses of AMD and then rinsed three times with a balanced salt solution. Untreated cells were then plated onto these dishes. At doses up to 0.5 μg/ml, no reduction in plating efficiency was observed. However, at doses >1.0 μg/ml, a dose-dependent reduction in cell survival was seen.

Heat Treatment

Monolayers of cells in petri dishes were exposed to 43°C for varying intervals up to 2 hours by placing the dishes in specially designed hot-water baths in incubators (17). At the beginning of heat exposure, the cells were covered with 5 ml of Eagle's MEM (control) or Eagle's MEM mixed with AMD (drug); the pH was maintained between 7.2 and 7.4 by a regulated gas flow of a mixture of 5% CO_2 and 95% air. The temperature was maintained at ± 0.1°C throughout heat exposure. Control experiments were performed to demonstrate the stability of AMD at doses of 0.1–1 μg/ml after heat exposure at temperatures of 43°C for 2 hours.

Sequence

Simultaneous heat exposure involved heating cells to 43°C during exposure to AMD (0.5 μg/ml) for periods ranging from 30 to 120 minutes.

Pre-heat was defined as exposure of cells to heat at 43°C for periods ranging from 30 to 120 minutes immediately followed by exposure to AMD (0.5 μg/ml) at 37°C for 2 hours. To test the recovery time to drug sensitivity, recovery experiments were performed by pre-heating cells for 90 minutes at 43°C, followed by 37°C incubation for periods of time ranging from 2 to 24 hours, prior to 2-hour exposure to AMD at 0.5 μg/ml.

Post-heat experiments were performed by an initial 2-hour exposure to AMD (0.5 μg/ml) at 37°C followed by exposure to heat at 43°C for intervals of 15–120 minutes.

Labeled AMD Incorporation

Experiments were performed using ^{3}H-AMD (0.5 microcurie/ml; specific activity, 5.1 curies/mmol). The ^{3}H-AMD was mixed with cold AMD so that the total concentrations of drugs were 0.5 μg/ml in order to match the concentrations used in the survival experiments.

RESULTS

Simultaneous Heat Exposure

The survival response of cells after the simultaneous exposure to AMD at 0.5 μg/ml and heat at

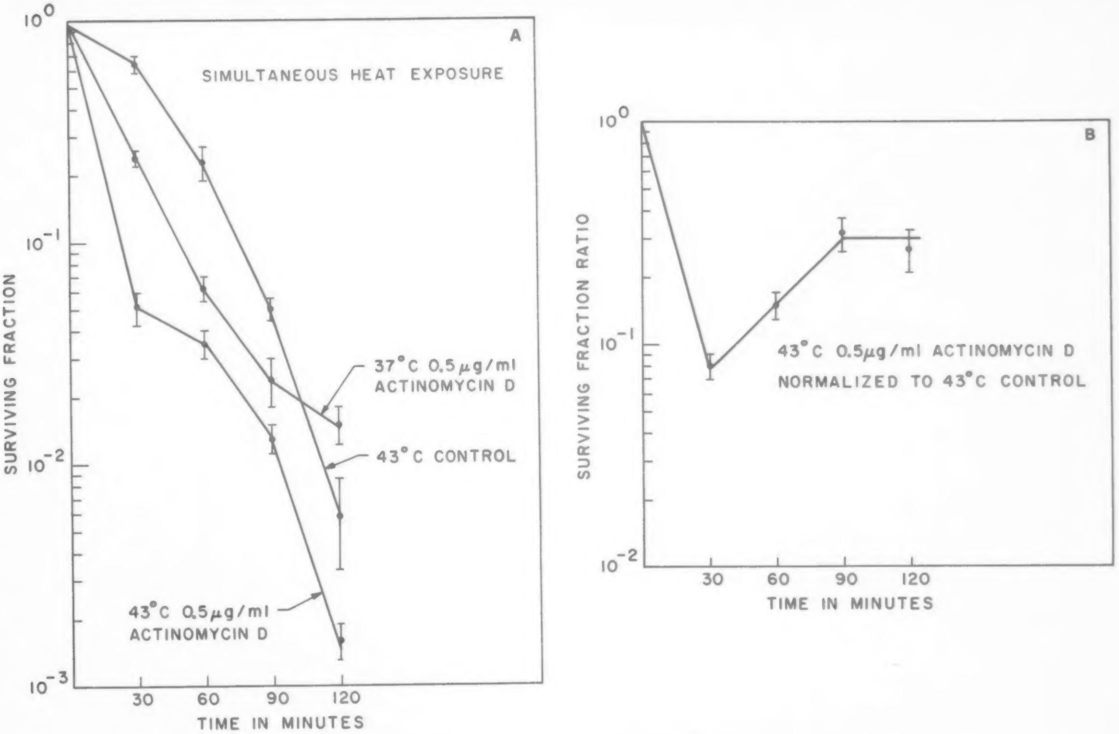

FIGURE 1.—Survival response of HA-1 cells exposed simultaneously to 43°C and AMD for various lengths of time. (A) Surviving fraction values of the combination of AMD and heat at 43°C as compared to controls of AMD at 37°C and heat alone at 43°C. (B) Surviving fraction ratios obtained by dividing, point by point, the values of the combination of AMD at 43°C by the 43°C control curve. The resulting curve is a measure of the cytotoxicity of AMD at 43°C. Error bars = ± 1 SE of the mean and define the accuracy of the assay derived from 2 replicate plates, each with 4 individual dishes/plate.

43°C for periods of time ranging from 30 to 120 minutes is shown in figure 1A for one representative experiment. Two controls were used in this experiment, as shown in figure 1A: the survival of cells exposed to AMD (0.5 µg/ml) at 37°C and that of heat at 43°C alone without added drug. The results from the control using AMD at 37°C showed an initial fast rate of cell-killing which slowed with time, perhaps as binding sites became saturated with drug; results from the 43°C heat control were identical to those from previous reports (8,17). The simultaneous exposure to heat plus AMD revealed appreciable cell-killing after 30 minutes of exposure with only 5% of the cells surviving. This enhanced cell-killing was contrasted to a predicted 15% level of survival which would be obtained if the effects of heat alone (65% survival) and AMD at 37°C alone (24% survival) did not involve any interaction. However, at intervals ≥ 90 minutes, the combination treatment curve essentially paralleled the curve for 43°C heat alone.

Figure 1B shows the cytotoxicity of 0.5 µg/ml of AMD at 43°C. This curve of surviving fraction ratios was obtained by dividing the combination treatment curve point by point by the heat curve, and thus normalizing the curve to the 43°C control. Beyond 30 minutes, AMD no longer contributed to cell-killing and, in fact, there was a progressive protection against AMD effect conferred by heat, demonstrated by a 20-fold increase in survival by 120 minutes. This resistance to AMD was not due to thermal inactivation of AMD, as control experiments demonstrated that after a 2-hour exposure at 43°C, AMD did not show reduced cytotoxicity for a subsequent exposure at 37°C.

Pre-heat Experiments

The effect of heating the cells at 43°C for varying lengths of time prior to the 2-hour exposure to 0.5 µg/ml of AMD at 37°C in two representative experiments is shown in figure 2. The results in figure 2A

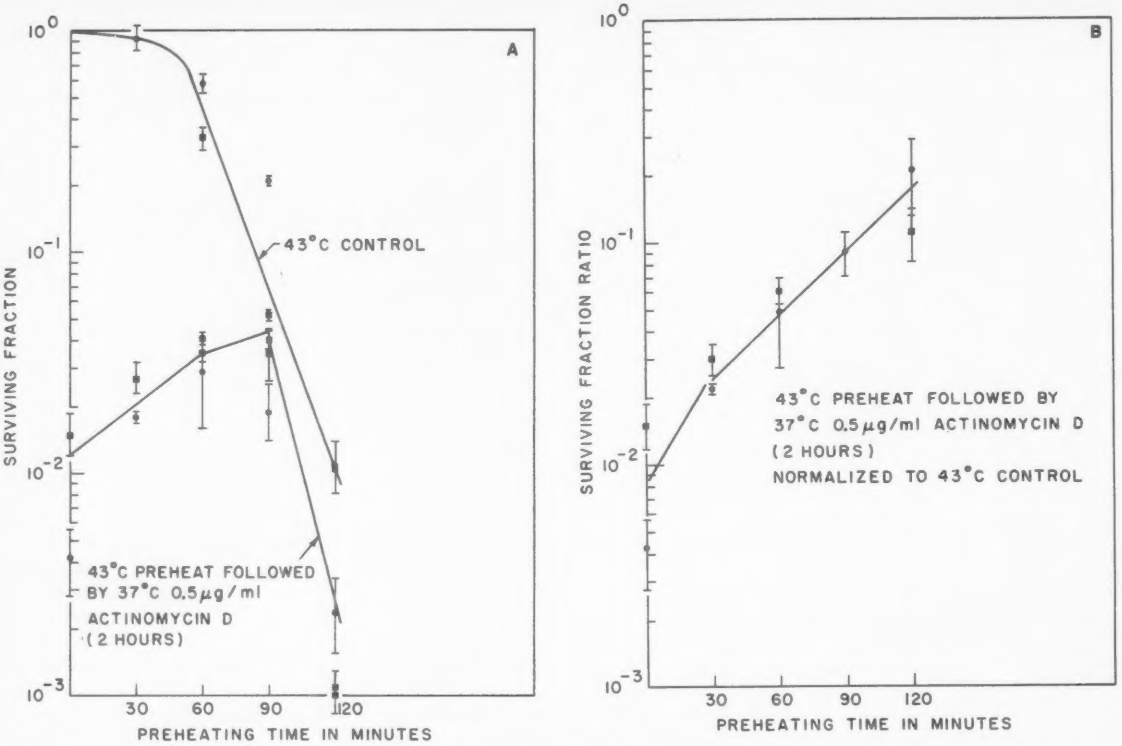

FIGURE 2.—Survival response of HA-1 cells pre-heated at 43°C for various lengths of time before exposure to AMD. Results of 2 experiments are shown by circles and squares. (A) Surviving fraction values for cells pre-heated at 43°C for periods of 30–120 mins followed by 2-hr exposure to AMD at 37°C as compared to 43°C alone. (B) Surviving fraction ratios obtained by dividing, point by point, the survival values obtained in the pre-heat experiment (43°C followed by AMD) by the appropriate 43°C control. This curve is a measure of the cytotoxicity of AMD at 37°C on cells pre-heated for the indicated durations. Error bars = ± 1 SE of the mean.

show a survival of approximately 1×10^{-2} for the unheated control (0-minute pre-heating) with a slight increase in the resistance to AMD up to periods of 90 minutes of pre-heat; thereafter, the curve is essentially parallel to the curve for 43°C heat alone. Thus, the heated cells became increasingly resistant to the cytotoxicity of AMD. This is illustrated in figure 2B which demonstrates the surviving fraction ratio of the cytotoxic effect of 43°C pre-heat at varying periods, followed by a 2-hour exposure to AMD at 37°C normalized to 43°C control. The surviving fraction ratio against preheating time is essentially linear, demonstrating an increased protection with longer periods of pre-heating, with a factor of 10 increase in surviving fraction ratio after 2 hours of pre-heating at 43°C.

To test how long the cells maintain their AMD resistance, a series of recovery experiments were performed. Cells were pre-heated at 43°C for 90 minutes, returned to 37°C for varying periods of time, and finally exposed to AMD at 0.5 μg/ml. The results of three such recovery experiments are shown in figure 3 plotted as surviving fraction ratio against recovery time. Surviving fraction ratio represents a surviving fraction for each of the time points divided by the surviving fraction of cells in AMD at 37°C with no pre-heating. These data show that 18 hours after pre-heating, most resistance to cytotoxicity of AMD is lost. Thus, after 18 hours cells have regained their sensitivity to AMD and survival of the pre-heated cells is approximately equal to that of the unheated control.

Post-heat

The effect of exposure to heat at 43°C for varying periods of time after a 2-hour exposure to 0.5 μg/ml of AMD at 37°C is shown in figure 4 for three representative experiments. Figure 4A shows that post-heating for periods of 30–60 minutes progres-

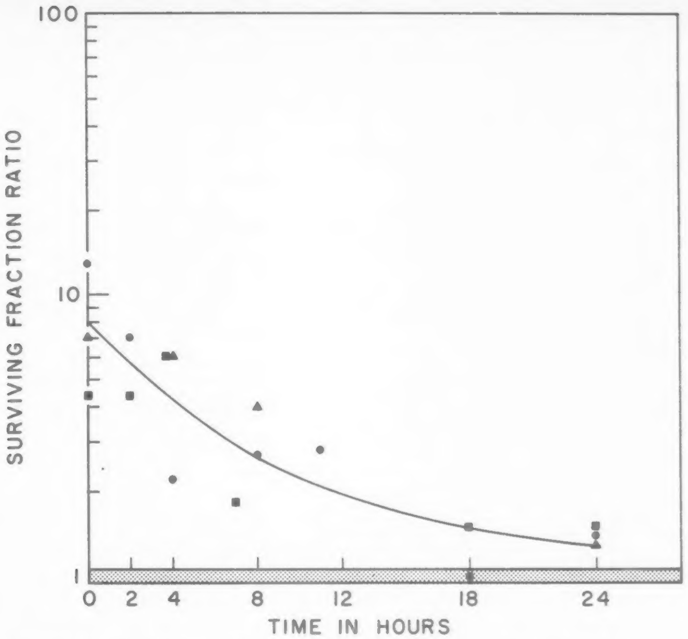

FIGURE 3.—Persistence of heat-induced AMD resistance. The ratio of survival values of cells heated (43°C, 90 mins) followed by variable "recovery" time at 37°C prior to exposure to AMD (0.5 μg/ml, 90 mins) is plotted against the recovery interval. Results of 3 different experiments are shown by squares, circles, and triangles. Complete return of AMD sensitivity (recovery ratio = 1) is reached by 18–24 hrs.

sively protects cells against the cytotoxic effects of a prior exposure to AMD (2 hours, 0.5 μg/ml at 37°C). Post-heating periods > 60 minutes have no further effect on AMD cytotoxicity. This is illustrated in figure 4B which shows the surviving fraction ratio of the cytotoxic effects of a 2-hour exposure of 0.5 μg/ml of AMD at 37°C followed by heating to 43°C and then normalized to 43°C control. We see here increasing resistance to AMD as shown by an increase in survival by a factor of 4–5 with 30–60 minutes of heat. This effect is not altered by prolonged post-heating beyond 60 minutes.

Labeled AMD Incorporation

Because induced resistance to AMD cytotoxicity suggests an alteration in the transport process of drug across the cellular membrane, we performed studies using ^{3}H-AMD with and without pre-heat (90 minutes, 43°C) (table 1). Our studies demonstrate that the mechanism for this induced AMD resistance is *not* a reduction in plasma membrane permeability by hyperthermia. In fact, more AMD enters across the plasma membrane into the intracellular compartment with pre-heating than without. However, this increase in drug is not relevant to cell-killing, for there was less killing of the pre-heated cells. Our studies demonstrate not only increased influx of AMD, but also an inability of the pre-heated cell to retain the drug, ie, an increase in drug efflux. The ^{3}H-AMD counts with and without pre-heat ultimately approach more or less the same level (table 1).

DISCUSSION

AMD has wide clinical use in a variety of adult and pediatric solid tumors. Since hyperthermia is now being extensively investigated in clinical trials as primary as well as adjuvant therapy, it is appropriate to study the interactions between the two modalities. In this study the timing of AMD plus hyperthermia was critical to the effect obtained.

AMD acts by intercalation with cellular DNA. Although other mechanisms have been postulated (6), hyperthermia is believed to kill primarily by

TABLE 1.—Comparison of cells treated with and without pre-heat*

Time after AMD exposure (mins)	cpm/10^6 cells	
	Cells exposed to ^{3}H-AMD at 37°C	Cells heated 90 mins at 43°C and then exposed to ^{3}H-AMD at 37°C
2	7451	15,450
60	6833	5964
120	4711	5295

*Cells (pre-heated or control) were exposed to ^{3}H-AMD (0.5 microcurie/ml; specific activity, 5.1 curies/mmol) for 120 mins. They were rinsed, fresh drug-free medium was added, and the cells were returned to 37°C incubation for 2, 60, or 120 mins. Counts retained were analyzed by liquid scintillation counting.

protein inactivation (18). Neither AMD nor hyperthermia is phase-specific, although hyperthermia does kill S phase and mitotic cells to a greater degree than G_1 cells (18). It is, however, unlikely that cell-cycle-associated events are responsible for our observations. Various hypotheses to attempt to explain our observations include:

(1) AMD is known to induce alterations in the physical properties of DNA (19) which could be further modified by prolonged heat, interfering with binding of drug by DNA.

(2) At 43°C the cellular plasma membrane is initially more permeable to an influx of AMD; however, with increased time after AMD exposure more drug leaks out of the cell.

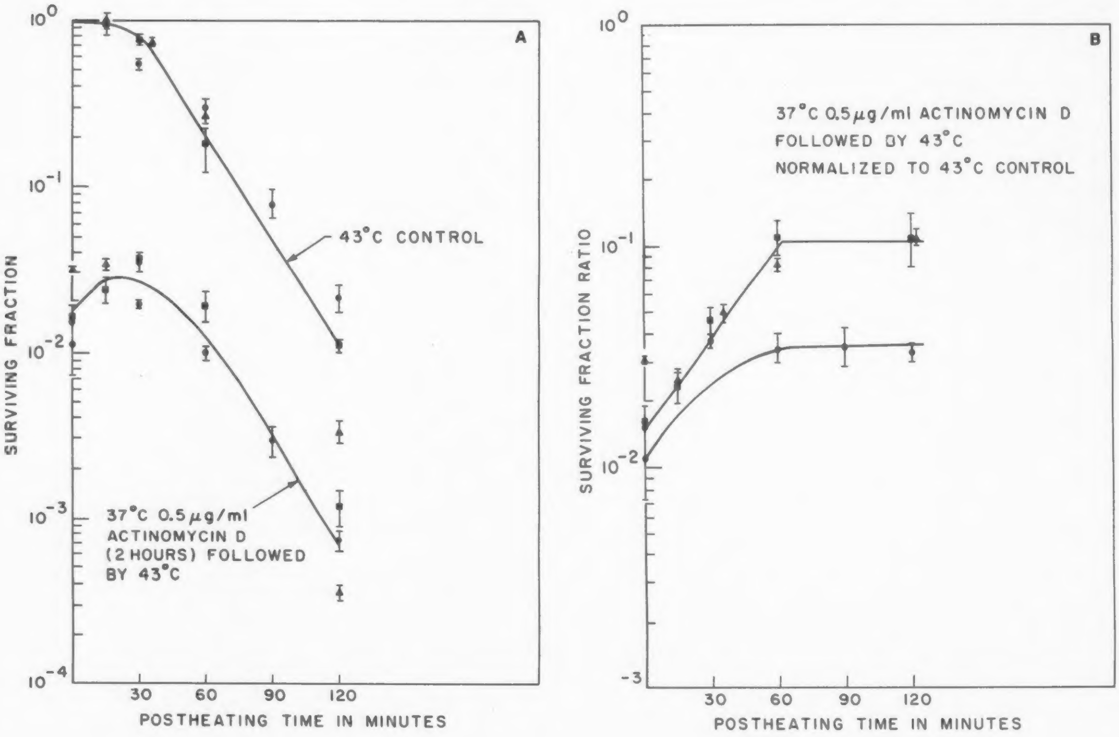

FIGURE 4.—Time response of post-heating HA-1 cells at 43°C following exposure to AMD in 3 experiments as shown in circles, triangles, and squares. (A) Surviving fraction values of cells exposed to 0.5 μg/ml of AMD at 37°C for 2 hrs followed by varying periods of post-heating ranging from 15 to 120 mins, as compared to heat alone at 43°C. (B) Surviving fraction ratios were obtained by dividing, point by point, the values of the 2-hr exposure of AMD at 43°C followed by post-heating by the heat-alone control (43°C). This curve is a measure of the cytotoxicity of AMD on cells post-heated for the indicated duration. Error bars = ± 1 SE of the mean.

(3) A short-term exposure to 43°C renders the cellular plasma membrane more permeable to influx of AMD as in point 2 above, but after 30 minutes the drug is no longer transported across the nuclear membrane.

The mechanism(s) involved for heat-induced drug resistance apparently cannot be explained by simple modulation of permeability, as was the case for adriamycin (8). The fact that post-heating also protects against AMD (fig 4) seems to rule out binding effects. Thus, our results favor hypothesis 2 above.

Our studies suggest that the resistance to AMD effect lasts approximately 18 hours. Thus, if AMD and hyperthermia are to be used in the same setting, we would hope to enhance their effect by increasing the time interval between heat and drug exposure to > 18 hours. In any case AMD does not appear to be a promising candidate for thermochemotherapy.

It would, however, be of clinical interest if preheating of localized areas could protect against the "recall phenomenon," observed when AMD is used following localized irradiation. This phenomenon, which is poorly understood, involves a reactivation of latent radiation effects by subsequent courses of AMD. It is most commonly observed in tissues such as the skin and mucous membranes. It might be possible to selectively and mildly heat certain irradiated tissues prior to systematic AMD administration. Heat-induced AMD resistance might then prevent the "recall phenomenon," a syndrome often associated with pain, discomfort, and morbidity. This hypothesis requires testing in animal systems prior to clinical use.

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Phase I Clinical Evaluation of Anguidine[1,2]

William K. Murphy,[3,*] Michael A. Burgess,[3] Manuel Valdivieso,[3] Robert B. Livingston,[4] Gerald P. Bodey,[3] and Emil J Freireich[3]

SUMMARY

Anguidine, one of a new class of agents derived from fungi, was given to 39 patients in a phase I study. The drug was administered by rapid iv infusion daily for 5 days at 2-week intervals, starting at a dose of 0.1 mg/m²/day. At $\geq$ 2.4 mg/m², this toxicity included nausea, vomiting, hypotension, central nervous system symptoms (including somnolence, confusion, and ataxia), diarrhea, chills and fever, generalized burning erythema, stomatitis, shortness of breath, moderate myelosuppression, and other toxicity of minor significance. An association of life-threatening toxicity with the presence of liver metastases or impairment of liver function was recognized at doses $\geq$ 5 mg/m². Subsequently, patients with known liver disease were initially given a lower dose. None of the 35 patients with solid tumors nor the four patients with acute leukemia had complete or partial remission, but only 15 patients were considered to have had treatment at adequate dose levels. The recommended dosage for phase II trials is 3.0 mg/m²/day for 5 days for patients with liver metastases or impairment of liver function and 5.0 mg/m²/day for 5 days for patients with normal livers; courses should be given by rapid (30–60 minutes) iv infusion every 3 weeks. Close observation should be made of renal and liver functions and hematologic parameters. For the present, patients with cardiovascular instability, jaundice, or ileostomy should be excluded from study with this drug.

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Anguidine, a sesquiterpene (diacetoxyscirpenol), is one of a new class of agents derived from fungi. It is the principal phytotoxic metabolic product of the plant parasitic fungus *Fusarium equiseti* and has also been isolated from culture filtrates of *F. diversiporum*, *F. sambucinum*, *F. scripi*, and *Gibberella intricans* (1,2).[5] It is related to the verrucarins Roridin A, Trichodermin, Nivalenol, and Fusarenon-X. The chemical structure of anguidine is depicted in figure 1 (3).

The basis for selection of anguidine for phase I studies was its activity in the P388 leukemia system. Given by ip injection on a continuous schedule (daily for 9 days) to mice bearing P388 leukemia, anguidine treatment resulted in a reproducible increased lifespan (ILS) of > 100%. Activity was also demonstrated in the L1210 leukemia system but 50% ILS was not achieved in all tests. Only slight activity was observed in the ip implanted B16 melanoma system, and there was no activity in the im implanted Lewis lung system. Activity has since been demonstrated against Line 38 colon adenocarcinoma in mice, as has synergism with 5-fluorouracil in the same model (4). These data were not available at the time of the initiation of this phase I study and had nothing to do with the selection of anguidine for clinical trials initially. No analogs of this drug have been previously tested clinically.[5]

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[3] Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

[4] Department of Medicine, The University of Texas Health Science Center at San Antonio.

**Reprint requests to:* William K. Murphy, MD, Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, Tex 77030.

[5] Helman L, Henney J, and Slavik M. Clinical brochure: anguidine. Prepared by the Division of Cancer Treatment, National Cancer Institute, Bethesda, Md, Mar 1976.

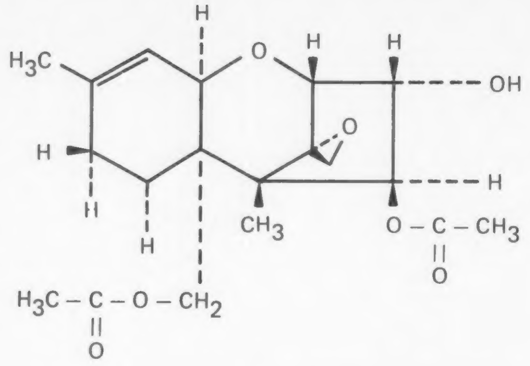

FIGURE 1.—Structural formula of anguidine (*from* ref 3).

Qualitative toxicity in beagle dogs receiving the toxic dose high (5 mg/m^2 × 1 day or 2.5 mg/m^2/day × 5 days) included emesis, polydipsia, congestion of the sclerae, hypoactivity, ptyalism, tremors, diarrhea, circulating nucleated rbcs, leukopenia and neutropenia, lymphopenia, anemia, and mild thrombocytopenia. In addition, there were increased levels of serum alkaline phosphatase, SGOT, SGPT, chloride, and BUN. All abnormalities were reversible and there was no anatomically or microscopically visible liver damage in killed animals. Lethal doses (5 mg/m^2/day × 5 days) produced essentially the same toxic effects noted above, plus ataxia. Pathologically, there was severe congestion of the spleen with lymphoid hypoplasia, severe necrosis and hemorrhage of Peyer's patches in the colon, moderate congestion with mucosal lymphoid infiltration of the stomach, lymph node hemorrhage, and congestion in all other organs. Effects on the hematopoietic system appeared to be generally dose-related and were more consistent in the dog than in the monkey. In dogs and monkeys that died secondary to therapy, no effects on the liver or kidneys were found except for congestion (5–9).[6] The mode of action of anguidine is thought to be irreversible blockage of protein synthesis (3).

Because of the potential usefulness of anguidine in the clinic, it was the purpose of this study to determine the maximally tolerated dose and toxicity associated with the administration of this compound to man.

MATERIALS AND METHODS

Adult patients with histologic proof of malignancy who were not candidates for known regimens or protocol treatments of higher efficacy or priority with disease refractory to prior treatment were considered eligible for this study. An estimated life expectancy of at least 10 weeks was required but the presence of measurable lesions was not a criterion. All patients had received previous chemotherapy, immunotherapy, and/or radiotherapy which had been discontinued for at least 4 weeks prior to being entered in the study, and all patients had recovered from all toxic effects of such prior therapy. For the small number of patients with acute leukemia, this requirement was waived. There was no age restriction, but all patients except those with acute leukemia were required to have adequate bone marrow function (defined as a peripheral absolute granulocyte count of $> 2000/mm^3$ and a platelet count of $> 100{,}000/mm^3$ with normal bone marrow cellularity). Adequate liver function (bilirubin of < 2.0 mg/100 ml) and adequate renal function (serum creatinine of < 2.5 mg/100 ml) were also required. Informed consent indicating the investigational nature of the study was obtained from all patients according to institutional policy. Complete remission was defined as a disappearance of all clinical evidence of active tumor for a minimum of 4 weeks, and partial remission was defined as a $\geq 50\%$ decrease in the sum of the products of the diameters of all measurable lesions without the appearance of any new lesions.

Anguidine was originally supplied in 20-ml vials containing 10 mg of anguidine as a white lyophilized powder plus 20 mg of mannitol. More recently it has been supplied in 5-mg vials with 100 mg of mannitol, and in this case it may be reconstituted with 10 ml of sterile water for injection (USP). It contains no preservatives and is stable for 24 hours after preparation. In this study it was administered in 100 ml of 5% dextrose in water iv over 30–60 minutes. The treatment schedule was daily for 5 days every 2 weeks.

The initial dosage and dose schedule were based on animal experimental data obtained from the P388 leukemia system, in which the best results were consistently obtained with schedules of multiple-dose administration, specifically by ip injection of the drug once daily for 9 days. The same was true in general for response in the L1210 leukemia system. The recommended dosage was based on the toxicity rating for anguidine in experimental animals. The highest nontoxic dose in beagle dogs for a daily × 5 dose administration schedule was 0.32 mg/m^2. One third of this dose was 0.1 mg/m^2/day × 5 days.

Escalations of 50%–100% were made until clinical evidence of toxicity was encountered. At least three patients were studied at each dose level and were evaluated for at least 2 weeks before additional

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[6] *See* footnote 5.

TABLE 1.—Anguidine dose levels

Dose (mg/m²/day × 5)	Initial dose		Subsequent dose	
	No. of patients	No. of courses	No. of patients	No. of courses
0.1–0.4	11	14	3	3
0.6–1.0	2	2	7	8
1.2–2.4	3	3	7	7
3.0–7.5*	23	39	6	8

*Includes patients starting at this dose range but having incomplete courses.

TABLE 2.—Patient population and characteristics

No. of patients entered in the study	39	
Men	23	
Women	16	
Median age (range)	52 yrs (18–70)	
Diagnosis	*Total No. entered*	*No. receiving ≥ 3 mg/m²*
Adenocarcinoma, colon	11	6
Adenocarcinoma, lung	1	0
Adenocarcinoma, pancreas	1	0
Adenocarcinoma, salivary gland	3	1
Adenocarcinoma, kidney	2	1
Adenocarcinoma, unknown primary	2	1
Carcinoma, adenocortical	1	1
Fibrous histiocytoma	1	1
Leukemia, acute myelocytic	3	1
Leukemia, lymphomatous	1	0
Melanoma	3	0
Sarcoma, myxoid	1	0
Lymphoma (mixed), nodular	1	0
Osteosarcoma	1	0
Squamous carcinoma, esophagus	1	0
Squamous carcinoma, lung	3	3
Squamous carcinoma, unknown primary	2	0
Malignant neoplasm, poorly differentiated	1	0

patients were started on an escalated dose level. Initially, to avoid cumulative toxicity, no dose escalation for an individual patient was permitted until the time course selected for repeated administration could be deemed satisfactory (table 1).

Each patient had a complete history and physical examination prior to entry in the study. Required laboratory studies included cbc count with differential and platelet counts, SMA–12 chemistry battery screen, urinalysis, electrocardiogram, chest roentgenogram, and, frequently, bone marrow examination. Additional radiologic studies and radionuclide scans were performed as clinically indicated before and during treatment. If possible, blood cell counts were obtained twice weekly while the patient was in the study, and SMA–12 screen and urinalysis were repeated prior to each course or more frequently as indicated. Treatment was continued until there was evidence of progressive disease or unless there was an unacceptable status quo.

RESULTS

Thirty-nine patients were entered in this phase I study of anguidine. Their characteristics are shown in table 2. All of the patients had received prior chemotherapy, usually more than one agent, and a number had been given radiation therapy. The number of patients entered at each dose level of anguidine is shown in table 1. Most patients were entered at daily doses of 3.0–7.5 mg/m², particularly at the dose of 5 mg/m².

Toxicity

Although all 39 patients were evaluable for immediate toxicity, three patients died within 1 week of starting treatment and an additional three patients returned home immediately following completion of their first course of treatment and refused further followup. No drug-related toxicity was noted in any patient receiving < 2.4 mg/m²/day × 5 days of anguidine. At the 2.4-mg/m² dose, only one patient had mild nausea and vomiting. Significant toxicity was seen only at levels of ≥ 3 mg/m². The side effects seen in the 25 patients receiving these doses of anguidine (≥ 3 mg/m²) are detailed in table 3. Nausea and vomiting of a moderate to severe degree were experienced by all patients. Other immediate side effects included diarrhea, somnolence (and/or mental confusion), fever and chills, generalized erythema with a burning sensation, hypotension, dyspnea, stomatitis, hives, and ataxia. After a single dose of anguidine at 5 mg/m², one patient who had undergone total colectomy for adenocarcinoma of the colon experienced moderately severe nausea and vomiting accompanied by massive ileostomy drainage and refused further therapy. The hypotension encountered was mild to moderate, was associated with no morbidity, and

TABLE 3.—Anguidine toxicity at doses ≥ 3 mg/m^2 in 25 patients

Toxicity	No. of patients (%)
Nausea and vomiting	25 (100)
Myelosuppression*	11 (79)
Hypotension	5 (20)
Somnolence (central nervous system symptoms including ataxia)	5 (20)
Diarrhea†	5 (20)
Fever and chills	3 (12)
Generalized "burning" erythema	3 (12)
Stomatitis	2 (8)
Shortness of breath	2 (8)
Other‡	3 (12)

*Only 14 patients were evaluable for myelosuppression.

†Includes 1 patient with ileostomy who had massive drainage.

‡1 patient each with total-body alopecia, urticaria, and phlebitis.

responded well to fluid administration. No patients required pressor therapy.

After several patients had been entered at the 5-mg/m^2 dose, it became apparent that some of them had much more severe reactions to anguidine than others. Characteristic of this toxicity was moderately severe nausea and vomiting, generalized burning erythema, hypotension, mental confusion, and/or somnolence in varying combinations. These side effects occurred after only one to three doses and were severe enough for discontinuation of therapy. On further evaluation, the severity of symptoms was found to correlate significantly with the presence or absence of liver metastases, elevated bilirubin levels, or liver function abnormalities (table 4). Subsequently, patients with liver abnormalities, defined as hepatomegaly (> 12 cm as determined by palpation, percussion, and/or liver scan), positive liver scan, liver enzyme abnormalities (alkaline phosphatase, LDH, or SGOT of $\geq 50\%$ above normal), and/or bilirubin > 1.5 mg/100 ml, received treatment at 3 mg/m^2/day × 5 days. Patients with normal liver size, scan, and function received 5 mg/m^2/day × 5 days. This eliminated the most severe toxic reactions previously seen.

After receiving two doses of the first course of anguidine at 5 mg/m^2, one patient developed urinary abnormalities, including hematuria, proteinuria and casts, along with elevation of BUN, creatinine, and uric acid levels. Treatment was discontinued at this point and all abnormalities disappeared within a few days.

TABLE 4.—Anguidine toxicity related to liver status (dose = 5 mg/m^2/day)

Toxicity	Abnormal liver	Normal liver
No. of patients (courses)	13	8
Nausea and vomiting	13*	8
Hypotension	4	1†
Central nervous system symptoms	4	1†
Generalized burning erythema	3	0

*Gastrointestinal toxicity was qualitatively more severe in all patients.

†Patient with probable leukemia liver infiltrate.

Anguidine was administered to 25 patients at doses of ≥ 3 mg/m^2 (no myelosuppression was seen at doses of < 3 mg/m^2). Of these, 14 were evaluable for myelosuppression and 11 were not. The reasons for inevaluability included shortened drug courses (due to other toxicity) and insufficient data. Of the 14 patients evaluable for myelosuppression, 11 had mild to moderate myelosuppression and three had none. The myelosuppression experienced by these patients is characterized in table 5. Of ten patients evaluable for course 1, only five had any myelosuppression and only two had thrombocytopenia. The median day for the lowest recorded counts was Day 12 for course 1 and Day 13 for course. 2. Three patients evaluable for three courses had myelosuppression in course 3, and three evaluable for the fourth course had no myelosuppression, probably a consequence of dosage adjustments. One patient had ten courses of anguidine at 5 mg/m^2 at varying intervals. Eight of these courses were evaluable for myelosuppression. For three courses there was no myelosuppression. The median interval between courses for this patient was 20 days (range, 16–29 days). For two patients, toxicity attributable to anguidine probably contributed to death. Both patients had significant liver disease. One of the treated patients had cardiovascular instability and was in a terminal state of disease. The other patient had the severe but not directly fatal reactions described previously as associated with liver disease.

Response

While the major thrust of this study was to evaluate the dosage and toxicity of anguidine, observations were also made regarding antitumor activity. The standard criteria for response were observed. A patient was considered evaluable for

TABLE 5.—Myelosuppression by treatment course*

Course No.	No. of patients	Median count × 10^3/mm^3 (range)			Median day of lowest count (range)
		Wbcs	Neutrophils	Platelets	
1	10	2.9(2.0–3.1)	1.5(1.2–2.6)	—(19 and 107)	12(8–14)
2	10	2.9(0.9–4.2)	1.8(0.5–3.1)	76(21–106)	16(6–29)
3	3	2.9(1.2–3.1)	1.7(0.4–1.8)	135	6(5–35)

*All data are for 5 mg/m^2/day × 5 days. Myelosuppression was seen at 3 mg/m^2 in 1 of 3 patients and at 4 mg/m^2 in 2 of 2 patients. The patient given 7.5 mg/m^2 had acute leukemia, and myelosuppressive data are inevaluable.

response if a minimum of two courses were administered and if the patient was followed for a total of 4 weeks. Patients with unequivocal progression (> 25% progression) after one course (2 weeks) were also considered evaluable. No objective tumor regression was noted in any of the patients entered in the study. However, a patient with adenocarcinoma of the colon had stabilization of previously progressing disease lasting for 6 months, and one patient with acute myeloblastic leukemia had a significant reduction of circulating leukemic cells on two occasions with no effect on the bone marrow.

DISCUSSION

With doses of anguidine < 3.0 mg/m^2 × 5 days by rapid (30–60 minutes) iv infusion, no evidence of physiologic activity was encountered. At that dose and greater, a variety of side effects were noted, including nausea, vomiting, fever, chills, somnolence, ataxia, generalized erythema with a burning sensation, hypotension, shortness of breath, diarrhea, stomatitis, total body alopecia, hives, and myelosuppression. Some patients also experienced weakness. The most striking side effect, the diffuse erythema and burning sensation, was most commonly found, along with several other side effects, in patients with significant liver abnormalities. The incidence of such severe, nonmyelosuppressive side effects was virtually eliminated by giving patients with liver abnormalities an initial dose of 3.0 mg/m^2 × 5 days.

Myelosuppressive toxicity of mild to moderate degree was observed only at doses ≥ 3 mg/m^2. Among patients receiving these doses, five of ten patients had myelosuppression following the first course, whereas eight of ten patients had myelosuppression following the second course. This suggests the possibility of cumulative toxicity with repeated treatment courses of anguidine. Maximum myelosuppression was observed 12 days after the initiation of treatment with the drug, suggesting that the interval between courses should be increased.

The absence of specific antitumor activity seen in this phase I trial may reflect the relatively small number of patients studied. Certainly, no definite conclusion can be made regarding antitumor activity, and phase II studies are indicated to evaluate the compound for possible antitumor activity in man. The activity of anguidine in transplanted colon tumors (Line 38 colon adenocarcinoma) in mice and its striking synergism with 5-fluorouracil, as reported by Corbett and Griswold (4), suggest that adenocarcinoma of the colon in man might be an exciting study to start with.

On the basis of this study, an initial dose of 5 mg/m^2 by intermittent iv infusion (50–100 ml of 5% dextrose in water iv over 30–60 minutes) daily for 5 days is recommended for patients with normal liver function. For patients with known liver metastases, liver enzyme abnormalities of ≥ 50% elevation, or mild bilirubin level elevation (< 1.5 mg/100 ml), an initial dose of 3.0 mg/m^2 for 5 days is recommended. Treatments should be administered at an interval of approximately 3 weeks. Patients with hyperbilirubinemia (> 1.5 mg/100 ml) should be treated with extreme caution until more experience is obtained with this agent. Patients with ileostomies probably should be treated only on an individual basis in the hospital. Patients with cardiovascular instability should not be treated with anguidine because of its potential hypotensive effects.

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An Enzymatic Technique for Measuring *N*-Phosphonacetyl-L-Aspartic Acid in Tissues[1]

David A. Cooney,* Mitchell G. Karlowicz, José Cubillan, Martha Roettger, and Hiremagalur N. Jayaram[2]

SUMMARY

An enzymatic technique is presented for measuring *N*-phosphonacetyl-L-aspartic acid (PALA; NSC-224131) in biologic specimens. Tightly bound PALA is quantitatively detached from its target enzyme, L-aspartic acid transcarbamylase (ATCase), by heating at 95°C for 5 minutes. Denatured proteins are removed by centrifugation. PALA in the supernatant fluid is quantitated by exposing intact splenic ATCase to representative aliquots or subdilutions of the resultant supernatant in the presence of L-[4-^{14}C]aspartic acid and carbamyl phosphate. After 30 minutes' incubation at 37°C, unreacted L-[4-^{14}C]aspartic acid is dissipated enzymatically and newly formed [4-^{14}C]carbamyl-L-aspartic acid is quantitated by scintillation spectrometry. The percentage inhibition of ATCase responds in a linear way to the logarithm of the concentration of PALA between 0.10 and 1.00 μM. The PALA concentration of an unknown is determined indirectly by matching the percentage inhibition caused by the unknown to the inhibition caused by a known series of standard concentrations of PALA over the linear range. This assay is sensitive, adequately reproducible despite the use of an unpurified enzyme, and notably facile. It can be used to measure PALA in plasma, urine, tissues, and tumors of subjects treated with this new oncolytic drug.

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N-phosphonacetyl-L-aspartic acid (PALA) is a potent inhibitor of L-aspartic acid transcarbamylase (ATCase) in vitro (1,2). PALA exhibits striking therapeutic efficacy against certain murine solid tumors in vivo (3,4). Phase I clinical trials of PALA started in early 1978. In order to permit pharmacologic disposition studies with the agent, a method for measuring PALA in physiologic specimens has been developed. This assay is based on the fact that there is a linear relationship between the logarithm of the concentration of PALA between 0.10 and 1.0 μM and inhibition of ATCase.

MATERIALS AND METHODS

Reagents

L-[4-^{14}C]aspartic acid (specific activity, 19.5 microcuries/μmol) was purchased from Amersham Searle Corp, Arlington Heights, Ill. The dilithium salt of carbamyl phosphate was the product of Boehringer Ingelheim Ltd, Elmford, NY. [^{14}C]PALA disodium salt (specific activity, 1.46 microcuries/μmol) was provided either by Dr. Marjorie Chadwick of Arthur D. Little, Cambridge, Mass, or Dr. Robert Engle of the National Cancer Institute, Bethesda, Md. The disodium salt of PALA was provided by the National Cancer Institute. ATCase purified from *Escherichia coli* (specific activity, 266.7 IU/mg of protein at 25°C) was a generous gift of Dr. E. R. Kantrowitz of Harvard University, Boston, Mass (5). All other reagents were commercially available and of analytic quality.

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[2] Division of Cancer Treatment, National Cancer Institute, Bethesda, Md.

**Reprint requests to:* Dr. David A. Cooney, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bldg 37, Rm 5B22, National Institutes of Health, Bethesda, Md 20014.

Preparation of Tissues

Mice were treated with a single ip injection of PALA, and in some cases with [^{14}C]PALA at the doses specified, or with physiologic saline, and were killed 24 hours thereafter by cervical dislocation. Tissues were excised, frozen on solid CO_2, and homogenized in a medium consisting of 5% (vol/vol) glycerol, 30% (vol/vol) dimethyl sulfoxide, 0.1 M Tris HCl (pH 8.4), 0.2 mM EDTA, and 2 mM dithiothreitol.

The homogenates were centrifuged at 12,000 × *g* for 3 minutes, and the clear supernatant was stored at –87°C for not > 1 week prior to further manipulation. Under these conditions PALA is completely stable. Denatured extracts were obtained by heating the organ homogenates at 95°C for 5 minutes followed by centrifugation at 12,000 × *g* for 3 minutes. In experiments involving the use of [^{14}C]PALA, radioactivity was measured in 5-μl aliquots of the resultant supernatant at 83% ± 3% efficiency. No quench corrections were made.

Crude splenic ATCase was prepared by homogenization of mouse spleen in 3 volumes (wt/vol) of the medium described above and centrifugation at 12,000 × *g* for 3 minutes. In some cases, this extract was dialyzed for 6 hours at 4°C versus 1 liter of homogenization medium.

Urine was collected in metabolism cages into receptacles cooled on dry ice.

Measurement of ATCase

At 4°C, the following reagents were added in sequence to Eppendorf 1500 μl polypropylene centrifuge tubes: 5 μl, 0.125 microcurie of L-[4-^{14}C]aspartic acid (adjusted with cold L-aspartic acid to a specific activity of 2.39 microcuries/μmol); 5 μl of 6.25 mM carbamyl phosphate in 0.05 M Tris HCl (pH 8.4) or 5 μl of the buffer alone; and 5 μl of splenic supernatant fluid prepared as described above. Five μl of denatured extract was added instead of intact splenic supernatant to triplicate vessels to act as controls for zero ATCase activity. The reactants were incubated at 37°C for 30 minutes (during which time the reaction was verified to be linear) and then heated at 95°C for 5 minutes. Unreacted L-[4-^{14}C]aspartic acid was then dissipated in the hood by enzymatic decarboxylation for 3 hours at 37°C in open vessels with 50 μl of a reagent consisting of 0.66 M sodium acetate buffer (pH 5.0) containing 6 mM α-ketoglutaric acid, 8.5 mM $ZnSO_4$, and 1 unit of L-glutamate-oxaloacetate transaminase. The reaction vessels were then immersed in scintillation vials containing scintillation fluid and counted at 83% efficiency in a Beckman LS-230 scintillation spectrometer.

Estimation of PALA

Aliquots of supernatant fluid from tumor or other tissues were heated at 95°C for 5 minutes and then centrifuged at 12,000 × *g* in the Eppendorf centrifuge for 3 minutes. Quadruplicate 5-μl aliquots of the resultant extract, or of serial halving dilutions thereof prepared in the homogenization medium, were added to Eppendorf vessels followed in order by 5 μl, 0.125 microcurie of L-[4-^{14}C]aspartic acid (specific activity, 19.5 microcuries/μmol); 5 μl of 3.125 mM carbamyl phosphate in 0.05 M Tris HCl (pH 8.4); and 5 μl of splenic extract to initiate the reaction. Included in each experiment as standards were a series of vessels in triplicate containing one of 15 serial $^3/_4$ dilutions of PALA at concentrations ranging from 0.024 to 3.2 μM. The assemblies were incubated at 37°C for 30 minutes, heated at 95°C for 5 minutes, and then subjected to decarboxylation and counting as described above. Under the conditions of the assay, with the splenic enzyme, a mean of 0.27 nmol of L-[4-^{14}C]aspartic acid (9400 dpm) is utilized in the absence of carbamyl phosphate; this is ~5% of the activity measured in the presence of carbamyl phosphate. PALA does not inhibit this "background" level of L-aspartic acid utilization.

Calculations

In the case of ATCase, the radioactivity in vessels lacking carbamyl phosphate was subtracted from the radioactivity in vessels receiving it, and the difference was taken as a measure of enzyme activity.

For the computation of inhibition, the activity of the enzyme in the presence of PALA was compared to activity in control vessels lacking the drug. In the case of standards, the concentration of PALA was plotted on the abscissa of semilog paper and the percent inhibition on the ordinate; unknowns were read from this diagram.

RESULTS

Figure 1 illustrates the inhibition by PALA of ATCase from spleen and *E. coli*. It can be seen that the enzyme from spleen is, under the conditions used, more sensitive to inhibition than the enzyme from *E. coli*. Nevertheless, over an empirically determined range, there is an approximately linear dose response with both enzymes: in the case of ATCase from *E. coli*, between 5 × 10^{-3} M and 5 ×

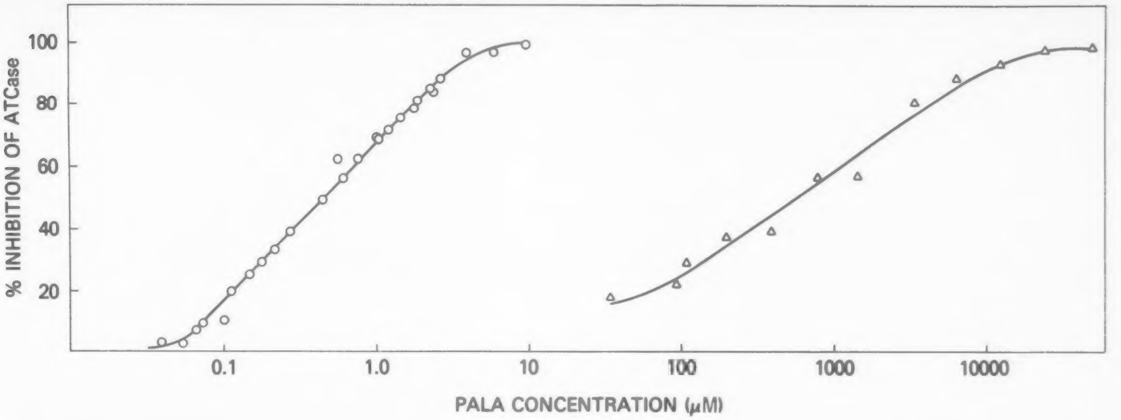

FIGURE 1.—Dose response of inhibition of ATCase by PALA. Various concentrations of PALA were incubated with ATCase from BALB/c spleen (○) or *E. coli* (△) and inhibition was measured according to the methodology described in the Materials and Methods section.

10^{-4} M; in the case of splenic ATCase, between 1 × 10^{-6} M and 1 × 10^{-7} M. A closely comparable dose response is seen when PALA is subdiluted in fresh splenic supernatant or in the homogenizing buffer (prepared as described in the Materials and Methods section) and the extracts are then subjected to denaturation at 95°C (data not shown). This finding suggested that precipitation of ATCase with heat quantitatively detaches PALA. This inference was fortified by the results of experiments with [^{14}C]PALA (table 1). In these studies quantitative detachment was produced by heating tissues and fluids at 95°C for 5 minutes. To explore the concentration dependence of recovery, experiments were conducted with 2.2×10^{-5} M, 2.2×10^{-6} M, and 2.2×10^{-7} M [^{14}C]PALA added to mouse splenic homogenate; total recovery of PALA was obtained, as measured both by the enzymatic and radiometric techniques, at all three concentrations of the drug. These results are also in accord with kinetic evidence which has established that the drug is a competitive (ie, non-covalent) inhibitor (6). It can also be concluded that PALA is stable to the elevated temperatures used in these manipulations.

The reproducibility of the radiometric technique presented here was studied by measuring the percent inhibition of splenic ATCase by PALA at four concentrations falling on the upper and lower part of the dose-response diagram. Thirty vessels were constituted for each of the four molarities examined. The percentages of inhibition (± SD) were 22.68% ± 1.22%, 31.64% ± 1.39%, 52.02% ± 2.75%, and 69.72% ± 4.88% at concentrations of PALA of 0.134, 0.178, 0.563, and 0.751 μM respectively. These values were significantly different ($P < 0.001$) from each other and from the uninhibited controls, a finding which is taken to indicate that the assay discriminates between closely spaced concentrations of PALA in an analytically satisfactory way.

Sources of Interference

Two potential drawbacks to the methodology presented here can be suggested. In most instances, for

TABLE 1.—Recovery of [^{14}C]PALA from tissues*

	% (± SD) recovery of added [^{14}C]PALA from heated-denatured extracts	
Tissue	Radiometric assay	Enzymatic assay
Mouse spleen	100.0 ± 1.5	100.0 ± 7.8
Mouse brain	100.0 ± 7.5	100.0 ± 12.4
Mouse liver	100.0 ± 2.7	113.6 ± 26.6
Mouse kidney	90.3 ± 1.0	100.0 ± 5.8
Mouse small intestine	95.5 ± 1.7	109.1 ± 11.9
Mouse pancreas	95.8 ± 8.9	98.2 ± 9.5
Human plasma	100.0 ± 9.7	90.7 ± 7.8
Human whole blood	97.2 ± 8.6	100.0 ± 10.6
Human urine	96.9 ± 4.5	100.0 ± 12.5

*Homogenates of the tissues were prepared in the medium described in the Materials and Methods section. To a 200-μl aliquot of the sample (tissue homogenate, plasma, whole blood, or urine), 5 μl of [^{14}C]PALA (250 nanocuries, 22.1 nmols) was added; an aliquot of this mixture was heated at 95°C for 5 mins and centrifuged at 12,000 × *g* for 6 mins; a 2nd series of aliquots was then taken for scintillation spectrometry and, after 100-fold dilution, for the enzymatic determination of PALA. Recoveries of [^{14}C]PALA of > 100% may be attributable to the volumetric disturbances arising from modest evaporation during the heating step and to the large protein pellet produced in this step which might have obligated a small amount of water.

TABLE 2.—Constancy of inhibition by PALA of ATCase from the spleens of 4 strains of mice*

Source of spleen (mouse strains)	% inhibition		
	(*PALA*)		
	2.37×10^{-6} M	1×10^{-6} M	0.42×10^{-6} M
BDF_1	82 ± 3	72 ± 3	59 ± 4
CAF_2	83 ± 3	70 ± 4	56 ± 4
C3H	79 ± 4	69 ± 3	58 ± 3
BALB/c	84 ± 3	68 ± 3	57 ± 3

*Inhibition of crude ATCase by PALA was carried out according to the methodology presented in the Materials and Methods section.

reasons of sensitivity, it uses a crude splenic enzyme, the characteristics of which might be variable from preparation to preparation. To examine this point, ATCase was extracted from the spleens of four different strains of mice and the response of the enzymes so extracted to graded concentrations of PALA was measured enzymatically. Table 2 shows that the percent inhibition, in the linear part of the dose-response diagram, is closely comparable with all preparations.

It could also be suggested that the use of crude supernatants from denatured extracts of tumors and tissues could introduce adventitious moderators of the activity of ATCase. Table 3 documents the finding that supernatants from the denatured homogenates of select tumors and tissues prepared according to the directions presented in the Materials and Methods section can, in fact, inhibit the ATCase activity of spleen by ~20% and also alter the degree of inhibition being exerted by PALA to a modest degree. Extracts of certain tissues, notably liver, also depress the inhibition of exogenous ATCase by PALA. These findings, which may be attributable to the influence in these extracts of L-aspartic acid, the second co-substrate, or other regulatory molecules, makes mandatory the inclusion of extracts from untreated mice in any study whose aim is the quantitation of the concentration of PALA in tissues.

Verification of the Method

To verify that the radiometric enzyme-inhibition assay for PALA was capable of providing a reliable index of the concentration of the drug in tissues, mice bearing subcutaneous Lewis lung carcinoma were given a single ip injection of saline or of [U-^{14}C]PALA (100 mg/kg) and were killed from 1 to 24 hours later; radioactivity and the concentration of PALA were then measured in the tumor according to the methodology outlined herein. Both types of analyses were in good accord (table 4).

TABLE 3.—Influence of addition of denatured tissue homogenates on the inhibition of splenic ATCase activity by PALA*

PALA concentration (μM)	% inhibition of splenic ATCase activity								
		Heat-denatured tissue extracts of—							
	Buffer	Brain	Gonads	Spleen	Liver	Thymus	Kidney	Lewis lung carcinoma	L5178Y/AR leukemia
1.33	76.3	67.5	71.4	78.8	64.6	74.9	74.6	—	—
1.00	63.0	67.8	66.4	71.9	57.8	73.7	66.1	—	—
0.75	58.6	59.9	61.7	68.8	51.2	62.6	62.6	—	—
0.56	52.5	54.6	60.1	62.2	41.6	57.1	66.2	48.5	54.7
0.42	47.0	40.3	47.8	45.8	36.7	46.8	47.4	—	—
0.32	33.0	30.2	38.5	43.9	28.0	42.3	43.3	—	—
0.23	31.6	31.3	35.5	35.6	20.5	33.1	32.4	—	—
0.13	12.0	—	—	—	—	—	—	11.0	9.5
0.0	0.0	17.8	15.3	16.6	10.4	20.7	16.6	—	—

*Extracts were prepared in the homogenization medium described in the Materials and Methods section and subjected to heating at 95°C for 5 mins. The enzyme inhibition assay with ATCase from spleen was conducted as described in the Materials and Methods section. The inhibition produced by the tissue extracts in the absence of PALA was dependent on the concentration of extract used and was abrogated by dilution.

TABLE 4.—Comparison of enzymatic and radiometric methods for the measurement of PALA in physiologic specimens*

Sample (hrs)	PALA concentration (μM) ± SD	
	Enzymatic method	Radiometric method
0.5	47.8 ± 7.5	50.7 ± 7.1
1.0	33.8 ± 8.4	34.9 ± 18.0
4.0	11.5 ± 1.2	11.8 ± 4.5
24.0	4.9 ± 0.3	5.1 ± 0.5

*BDF$_1$ mice bearing 10-day-old subcutaneous nodules of Lewis lung carcinoma were injected ip with 100 mg/kg (1.0 microcurie/mouse) of [^{14}C]PALA; tumors were removed at the stipulated time intervals, immediately frozen on solid CO_2, homogenized, and assayed for PALA and for [^{14}C]PALA according to the methodology described in the Materials and Methods section. For calculations, non-specific enzyme inhibition by tumor extract was determined in saline controls and used to correct the measured tissue levels in the experimental group.

DISCUSSION

Although many impure enzymes have been used as reagents, a purified enzyme or at least one of high specific activity is ordinarily desired for an enzyme-inhibition assay such as that described here. Indeed, the ATCase from Chinese hamster ovary cells resistant to PALA is an obvious candidate for such an analytic use,[3] inasmuch as it constitutes 10% of the soluble protein of the mutant cells and is readily purified to homogeneity (7). In the absence of this reagent, however, it would appear that ATCase from spleen is an attractive alternative for use in the type of analysis described here; it is present at high specific activity, is reasonably stable at -87°C in the medium used, and is outstandingly convenient.

Using this enzyme, it has been possible to quantitate the concentration of PALA in the principal viscera of mice given modest doses of the drug. The results of the study agreed well with a parallel study using [^{14}C]PALA. From such agreement one could hope that it will be possible to conduct studies with the methodology prescribed here during the clinical phase I trials of PALA.

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[3] In fact, experiments have been conducted with crude extracts of these Chinese hamster ovary cells and L5178Y leukemia. The slope of the dose response to PALA was identical to that seen with spleen. However, the percentage inhibition was depressed by 20% and 30% when leukemic and ovarian extracts, respectively, were used as sources of ATCase in lieu of spleen. Thus, the crude ATCases from leukemic and ovarian extracts provide a less sensitive assay for PALA.

Hepatic Arterial BCNU: A Pilot Clinical-Pharmacologic Study in Patients With Liver Tumors [1,2]

William D. Ensminger,* Madison Thompson, Steven Come, and E. Michael Egan [3]

SUMMARY

BCNU at a dose of 200 mg/m^2 was administered in a 60-minute hepatic arterial (HA) infusion to three patients with liver-predominant neoplastic disease. Nitrosourea blood levels were measured during the 60-minute infusion and for 30 minutes after completion of the infusion in samples obtained simultaneously from a hepatic venous (HV) catheter and a peripheral venous (PV) site. Direct extraction of blood with diethyl ether was used in order to remove quantitatively the nitrosourea from blood and thereby stabilize it against in vitro breakdown in blood prior to colorimetric analysis. As determined by blood levels, steady-state was achieved within 50 minutes of infusion. Mean HV levels at steady-state were 2.5-fold higher in the HV compared to PV samples. The higher HV compared to PV levels must reflect higher tumor exposure to nitrosourea with HA compared to the usual iv route of administration. No drug-related hepatic toxicity was noted. Myelosuppression was noted in only one patient in whom reversible leukopenia (granulocyte nadir, 500/mm^3) and thrombocytopenia (platelet nadir, 20,000/mm^3) developed.

[Cancer Treat Rep 62:1509–1512, 1978]

Regional infusion chemotherapy represents an attempt to improve the therapeutic index of a given agent by increasing the tumor drug exposure regionally without increasing the drug exposure of sensitive host tissues outside of the region of infusion. Most antitumor agents have a steep dose-response curve so that the higher the dose and/or concentration, the greater the antitumor and toxic effects. Hepatic arterial (HA) infusion therapy in patients with liver-predominant primary or metastatic tumor has been utilized most often with the fluorinated pyrimidines, 5-fluorodeoxyuridine (5-FUdR) and 5-fluorouracil (5-FU). The response rate and pharmacology of 5-FUdR and 5-FU in HA infusions suggest that a greater therapeutic selectivity is achieved (1–3). We have become interested in the use of BCNU in HA infusions due to its reported activity in colorectal cancer (4) and its pharmacologic properties, ie, a short serum half-life of 5–15 minutes and extensive hepatic uptake and hepatobiliary metabolism (5,6). This report summarizes a pilot clinical-pharmacologic study of HA BCNU in which hepatic venous (HV) drug levels were used as a minimal measure of tumor drug exposure.

METHODS

Three patients (two females and one male), 41–67 years old, with liver-predominant tumor from lung cancer, cholangiocarcinoma, and melanoma, were entered in the study. All patients had nodular enlargement of the liver documented by scan. One patient had a normal bilirubin, SGOT, and serum alkaline phosphatase. In the other two patients, the bilirubin was 1.5–2 times normal and the SGOT and alkaline phosphatase were 2–4 times normal. Clotting studies including the prothrombin time, partial thromboplastin time, and platelet count were nor-

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[3] Divisions of Clinical Pharmacology and Medical Oncology, Sidney Farber Cancer Institute, Boston, Mass.

**Reprint requests to:* William D. Ensminger, MD, Division of Hematology/Oncology, Simpson Memorial Institute, University of Michigan, 102 Observatory, Ann Arbor, Mich 48109.

mal in all patients. Written informed consent was obtained from all patients.

Patients were hospitalized at the Sidney Farber Cancer Institute, Boston, Mass. HA and HV catheters were placed through a femoral approach under fluoroscopic guidance and drug was infused while the patient remained in the radiology suite. Catheters were immediately removed after drug infusion was complete. A three-way stopcock was attached to the HV catheter for blood sampling and a 19-gauge scalp vein needle with stopcock was used for peripheral venous (PV) sampling.

BCNU was dissolved in ethanol and diluted 20–30-fold into normal saline for a final volume of 200–250 ml. Drug was put into solution in a glass bottle within 10 minutes of start of infusion. The solution of BCNU was infused at a dose of 200 mg/m² through the HA catheter using a constant infusion pump (IMED Corp, San Diego, Calif) over 1 hour with blood sampling from the HV and PV lines as noted in figure 1.

Due to the extreme lability of BCNU in biologic fluids, a method for rapid extraction of the nitrosourea was developed. Two-ml samples of blood were immediately added to 4 ml of chilled diethyl ether in stoppered glass tubes containing 15 mg of EDTA (Monoject blood collection tubes) with immediate shaking of the mixture and placement on ice. The lipophilic nitrosourea is thereby quantitatively extracted into the ether layer where it is stable. A standard curve was constructed by the addition of known amounts of BCNU to samples of the patient's blood drawn prior to the infusion. Standards were processed in the same manner as samples that were drawn during the infusion. In two patients the infusate was also sampled at the end of the 60-minute infusion to monitor potential drug breakdown in vitro. The ether layer was separated within several hours and stored at –10°C until further processing in the colorimetric assay below.

The colorimetric assay for nitrosourea uses the Loo and Dion modification (7) of the Bratton-Marshall reaction to determine the nitrous acid released from the nitrosourea by the treatment with HCl acid. Ether samples were evaporated to dryness under vacuum from a water aspirator on a rotary evaporator. One ml of water was added to each dried sample followed by 0.5 ml of sulfanilamide reagent (5 g of sulfanilamide/liter of 2 N HCl). After incubation at 50°C in a water bath with occasional shaking for 45 minutes, samples were chilled on ice. To chilled samples was added 0.1 ml of the Bratton-Marshall reagent (300 mg of *N*-[1-napthyl]-ethylenediamine hydrochloride in 100 ml of water). After 10 minutes of incubation at room temperature, the absorbancy at 540 mμ was read against a blank processed from the patient's serum which had no nitrosourea. The standard curve was linear in all instances over a drug concentration from 0.5 to 4.0 μg/ml. Figure 2 plots the mean (± 1 SD) standard curves from the three patients in the study. Nitrosourea concentration as determined by the colorimetric assay did not change by > 10% in vitro in the infusate bottle over the 60-minute administration.

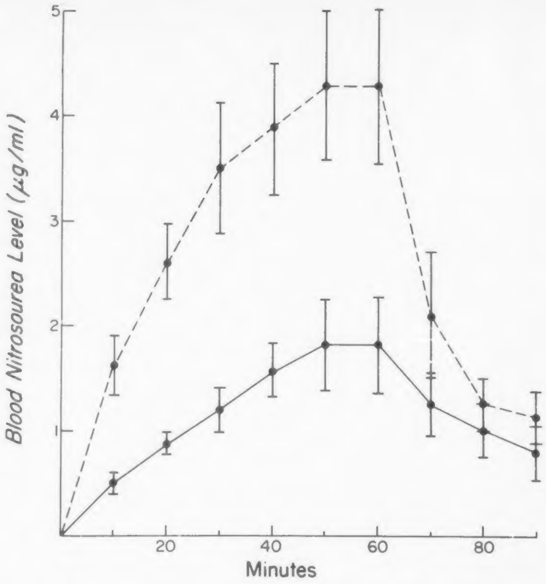

FIGURE 1.—Simultaneous HV and PV nitrosourea levels during a 60-min HA infusion of BCNU (200 mg/m²) and for 30 mins after the infusion has ended. Data points = mean ± 1 SD for the 3 patients studied. HV levels (·----·), PV levels (·—·).

RESULTS

The mean (± 1 SD) HV and PV nitrosourea levels obtained from a 60-minute HA infusion of BCNU are presented in figure 1. Levels rise steadily during the infusion until a steady-state appears to be reached by about 50 minutes. The steady-state PV nitrosourea level averages 1.8 μg/ml whereas the steady-state HV level is about 2.4-fold higher with a mean value of 4.3 μg/ml. This differential is maintained during the infusion and then rapidly dissipates within 20 minutes after the infusion has ended. The higher HV level at 70 minutes, ie, 10

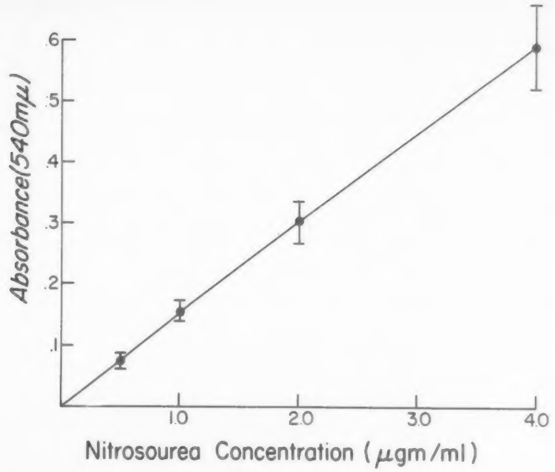

FIGURE 2.—Correlation of absorbance with nitrosourea concentration using known amounts of BCNU added to patient's blood with analysis as described in the Methods section. Data points = mean ± 1 SD on the 3 patients studied.

minutes after the BCNU infusion has ended, may represent release of nitrosourea (including colorimetrically active metabolites) from liver cells. Integration of the PV and HV concentration plots for a concentration × time (C × T) product over the 60-minute infusion and the 30 minutes thereafter demonstrates that the C × T for HV nitrosourea is 2.3-fold higher than the C × T for PV levels. As will be discussed later, the drug exposure (approximated by the C × T) for the hepatic tumor capillary bed is certainly higher and the HV/PV differential can only be used as a minimal estimate of improved selective drug exposure.

Patients were monitored for acute drug side effects during the HA infusion and for 24 hours thereafter. Weekly blood counts, liver function tests (SGOT, serum alkaline phosphatase, and bilirubin), and frequent physical examination were used to detect delayed toxicity. Acute drug side effects occurring during the infusion and rapidly subsiding after completion of the infusion included a generalized flushing of the skin, most prominent in the head and neck region. Patients consistently complained of a sensation of warmth associated with the flushing. Nausea and vomiting occurred in one patient at the end of the infusion and in all patients 4–6 hours later. Two of the three patients showed no myelosuppression or elevation of liver function tests on weekly followup. One patient developed myelosuppression with a granulocyte nadir of 500/mm^3 and a platelet nadir of 20,000/mm^3 5 weeks after infusion, but did not require supportive therapy. This patient maintained a normal bilirubin, but the SGOT and alkaline phosphatase gradually rose, becoming 2-fold higher than normal by 5 weeks after infusion. Unfortunately, the contribution of progressive liver involvement by tumor in this latter patient cannot be separated from possible drug-induced hepatic toxicity. None of the three patients developed symptoms suggestive of hepatocellular necrosis. Two other patients, who were not part of this pharmacologic study, have received 150 mg/m^2 of BCNU as a 1-hour HA infusion either prior to or after several weeks of constant HA infusion of 5-FUdR. Neither of these two patients receiving HA combination chemotherapy demonstrated any signs or symptoms of hepatocellular damage.

Although none of the three patients receiving BCNU alone showed significant clinical improvement, hepatic pain and tenderness were relieved for 4–6 weeks in two of the patients. However, all three of the patients had advanced disease and two of the patients had received extensive prior chemotherapy.

DISCUSSION

A major aim of HA infusion is to improve the therapeutic index of the chosen agent by increasing drug exposure for hepatic tumor without increasing exposure for sensitive host tissues such as bone marrow and gut. In many respects, the factors relevant to HA infusion are common to other regional arterial approaches. In particular, the computer modeling presented by Fenstermacher and Cowles (8) to examine drug distribution differences with regional arterial versus systemic venous drug infusions in brain tumor chemotherapy should be applicable to liver therapy as well. These analyses indicated that brain (and thus brain tumor) exposure was some 3-fold greater with intracarotid versus iv infusion for a drug with a half-life of 30 minutes. This differential increased when a drug with a shorter half-life was analyzed in the model. We have recently studied the selective pharmacologic advantage of 5-FUdR and 5-FU in HA infusions using drug blood level determinations on patients with primary or metastatic liver tumors (3). Although extremely high doses of 5-FUdR and 5-FU were necessary due to the insensitivity of the drug assays, nevertheless, HA infusion produced 2–4-fold greater regional drug exposure with one half to one fourth as much systemic exposure in comparison to peripheral iv administration (3). Unfortunately, the delayed and potentially severe myelosuppression of BCNU and the limited sensitivity of the BCNU colorimetric assay have precluded similar extensive studies with this agent.

Our results indicate at least a 2-fold increase in BCNU exposure for hepatic tumor with HA infusion. However, actual exposure should be considerably higher since most of the tumor blood supply generally comes from the hepatic artery and not the portal vein (9,10). Usually, about one third of the hepatic blood flow comes from the hepatic artery (9,10). In a short-term infusion, portal venous nitrosourea concentration is probably no greater than the PV concentration. Hence, portal venous blood of lower drug concentration mixes with HA blood of higher BCNU concentration to create HV blood with intermediate drug levels. In order to achieve the 2-fold higher HV drug level, HA and thus hepatic tumor capillary drug concentration should be at least 6-fold greater than portal venous (or PV) drug concentration. The drug concentration in the hepatic artery can be estimated in another manner using the rate of drug infusion (4–6 mg/minute) and the approximate rate of HA blood flow (200–600 ml/minute) into which the drug is diluted (3,9). This calculation produces a range of 7–30 μg/ml as the BCNU concentration in the hepatic artery during the drug infusion. The calculated value is approximately 2–7-fold higher than the HV levels which reach a maximum of about 4 μg/ml. Rapid iv administration of BCNU (150 mg/m^2) results in peak levels of nitrosourea that are slightly > 100 μM (11). Blood levels rapidly fall to 10–20 μM by 10 minutes after injection. Blood levels of 7–30 μg/ml such as are achieved in the hepatic artery by direct HA infusion approach the 100-μM levels transiently obtained by iv infusion. However, with HA administration this high level is maintained in the hepatic artery throughout the 60-minute infusion resulting in a much greater C × T for hepatic tumor. Systemic drug levels with HA infusion in this study reached a steady-state at 2 μg/ml (about 10 μM). Pertinent to this regional hepatic-systemic drug concentration gradient is the recent evidence that human bone marrow function as assayed by colony formation and ^{3}H-thymidine incorporation into DNA in vitro is not appreciably affected by short-term exposure to 10 μM nitrosourea, but is adversely affected by exposure to ≥ 100 μM levels (11).

Thus, we have shown that diethyl ether can be used to extract the otherwise unstable (5,6) nitrosourea directly from blood and that the colorimetric assay of Loo and Dion (7) is thereby of sufficient sensitivity to measure the nitrosourea moiety in short-term BCNU infusions. Furthermore, direct BCNU infusion into the hepatic artery leads to what is probably at least a 4-fold greater drug exposure for tumor without increased exposure for bone marrow and gastrointestinal tract. Although no significant antitumor effects were noted, there was no hepatic toxicity noted or other unusual toxicities associated with this route of administration. Based on previous clinical studies showing improved response rates in gastrointestinal carcinoma with nitrosourea and fluorinated pyrimidine combinations (4), it is reasonable to speculate that short-term HA infusion of BCNU combined with continuous HA infusion of fluorinated pyrimidines could potentially improve response in liver-predominant neoplasia.

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Acute Myelomonocytic Leukemia Following a Chemotherapeutic Regimen for Metastatic Sarcoma [1,2]

Ehsan Youness,* Gary Dosik, Robert S. Benjamin, and Jose M. Trujillo [3]

SUMMARY

Acute myelomonocytic leukemia developed in a patient 18 months after treatment with cyclophosphamide, vincristine, adriamycin, and DTIC, a chemotherapeutic regimen used for the treatment of metastatic sarcoma. The patient had had no prior history of radiation. More than 400 patients received this treatment and none of them developed leukemia. The occurrence of leukemia in this relatively short period of time may have been caused by the combined chemotherapeutic agents. However, confirmation of this will require long-term followup studies in cancer patients receiving chemotherapy to determine the true risk of second malignancies.

[Cancer Treat Rep 62:1513–1516, 1978]

Patients with primary malignant neoplasms have an increased risk for developing a second malignancy. In addition, the treatment of cancer often involves therapeutic modalities such as radiation therapy and chemotherapy which are, themselves, carcinogenic (1–6). It is, therefore, not surprising that several recent studies have emphasized the occurrence of a second malignancy in patients whose primary cancer has been controlled or cured with one or more therapeutic modalities (1,2,5–14).

Acute granulocytic leukemia has been reported in patients treated with alkylating agents and/or radiation therapy for malignant solid tumors, particularly ovarian (6–9) and breast (4) cancers. In addition, several studies have shown an increased incidence of this complication after treatment of Hodgkin's disease, especially when chemotherapeutic agents and radiation therapy are combined (1,2,5). We report here on a patient who developed acute leukemia 18 months after initially receiving chemotherapy for metastatic sarcoma.

CASE REPORT

A 64-year-old woman was referred to the University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, on November 24, 1975, for multiple pulmonary nodules. In 1973, she had uterine bleeding and an abnormal uterine curettage. An abdominal hysterectomy and bilateral salpingo-oophorectomy were done. A diagnosis of adenocarcinoma (grade I) with superficial myometrial invasion was made. A hemorrhagic leiomyoma was also noted in the specimen. The pathologic material was reviewed and confirmed by our pathologists.

A further workup at our hospital revealed a normal liver scan, bone scan, and bone marrow biopsy. Cbc, differential, and platelet counts were also within normal limits. In addition, a barium enema, an upper gastrointestinal series with a small bowel follow-through, and an iv pyelogram were normal. On December 9, 1975, the left thoracotomy with the resection of one tumor nodule was accomplished. The tumor was studied by light and electron microscopy. A diagnosis of metastatic sarcoma, strongly suggestive of leiomyosarcoma, was made.

The patient began treatment with a chemotherapy protocol consisting of cyclophosphamide, vin-

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[3] Departments of Laboratory Medicine and Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

**Reprint requests to:* Ehsan Youness, MD, Department of Laboratory Medicine, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, Tex 77030.

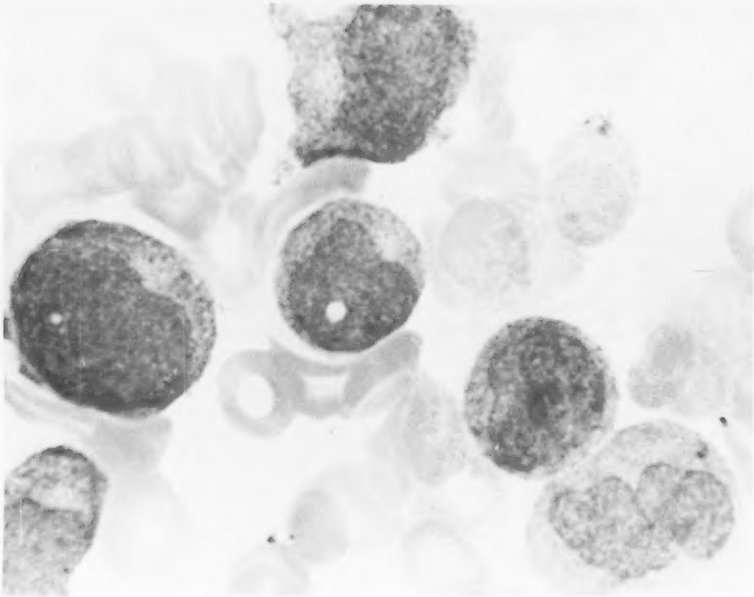

FIGURE 1.—Blast cells in bone marrow aspirate showing monocytoid features (Wright-Giemsa stain, × 800).

cristine, adriamycin, and DTIC (CyVADIC), a regimen developed at our institution for use in metastatic sarcomas (15). The first three courses were administered with step-wise dose escalation in a sterile protective environment while the patient was taking prophylactic nonabsorbable antibiotics. In March 1976, she was discharged in partial remission with maintenance therapy consisting of monthly courses of CyVADIC. The pulmonary nodules had regressed by $> 50\%$ prior to discharge from the hospital. In November 1976, the nodules began to increase in size. Despite continuation of the chemotherapy at increased doses, the tumor progression was unremittant. In February 1977, actinomycin D was substituted for adriamycin in the CyVADIC regimen. At this time, monocytosis was noted in a peripheral blood smear. The patient was re-evaluated in June 1977. A chest roentgenogram revealed progressive metastatic lesions in both lungs. She then received treatment with *cis*-dichlorodiammineplatinum(II) at a dose of 15 mg/m^2/day for 5 days in an attempt to control her metastatic disease.

A routine pretreatment bone marrow aspiration was obtained. The clot section revealed marrow particles with a cellularity of 70%. Sheets of immature myelomonocytic cells were seen. The megakaryocytes were normal. There was no evidence of metastatic tumor. The bone marrow smear differential revealed 50% blast cells which were medium to large and irregular, with a moderate amount of cytoplasm and no Auer rods. The nuclei were folded and had several nucleoli (fig 1). A battery of special stains revealed some of the immature myelomonocytic cells to be peroxidase positive; the majority were nonspecific esterase positive. A periodic acid-Schiff stain was negative. The cbc count revealed a hemoglobin of 12.5 g/100 ml, hematocrit of 38.4%, platelet count of 75,000/mm^3, and wbc count of 5800/mm^3. The differential smear showed 43% neutrophils, 29% lymphocytes, and 28% monocytes. The patient was diagnosed as having acute myelomonocytic leukemia.

The patient had no history of radiation or other toxic chemical exposure. A careful family history taken from the patient and various relatives revealed no history of neoplasia in the patient's children, siblings, parents, or grandparents.

Although a transient decrease in leukemic infiltrate was noted coincident with the first course of *cis*-dichlorodiammineplatinum(II), 3 weeks later the leukemia had clearly progressed. Intensive antileukemic therapy was instituted with cytosine arabinoside, vincristine, and prednisone without response. The patient died 2 months later of pulmonary infection and active acute leukemia.

DISCUSSION

This patient developed acute myelomonocytic leukemia 18 months after the diagnosis of metastatic sarcoma. A predisposition to various malignancies such as breast cancer, brain tumor, leukemia, and adrenal cortical carcinoma has been noted in sarcoma patients and their families (16–20). Although this might explain the occurrence of three distinct cancers in our patient, a carefully taken history revealed no other incidence of neoplasia in this patient's family over a total of four generations. In addition, bright-field microscopy of normal and malignant tissue did not disclose intranuclear cytoplasmic invaginations, intranucleolar bodies, or acidophilic intracytoplasmic inclusions variably associated with familial syndrome (20).

Thus, leukemia may represent a late complication of chemotherapy for metastatic sarcoma. A second malignancy complicating treatment of lymphoma with radiation therapy and combination chemotherapy has been reported to be 18–27 times that expected (1,2). Similarly, in a review of 680 patients with Hodgkin's disease whose survival was > 1 year, Coleman et al found the incidence of hematologic neoplasia to be 2.9% for the entire group and 3.9% for 330 patients treated with combination radiation and chemotherapy (5). The observed to expected ratio for patients treated with chemotherapy alone was 262 whereas for patients who also received radiation therapy it was 1333. Reimer et al studied > 5000 patients with ovarian cancer who had received alkylating agents; the relative risk in 3-year survivors was 171.4 (6).

The carcinogenicity of alkylating agents has been associated with their ability to produce chromosomal damage (21–24). Since radiation can cause similar lesions the effect of both may be additive (23). Our patient received no ionizing radiation. She did, however, receive adriamycin in combination with an alkylating agent. This combination has been demonstrated to be additive or synergistic for tumors in experimental animals and man (24,25). This synergism extends to cardiotoxic effects (26) and may, therefore, also include carcinogenesis as well. The addition of DTIC whose active moiety is an unstable carbonium ion (27) may also serve to increase the likelihood of a second neoplasm. Since cancer chemotherapeutic agents also have immunosuppressive properties, it has also been suggested that these drugs may contribute to oncogenesis through immunosuppression (28), direct oncogenic effect, potentiation of environmental carcinogens, or allowance of oncogenic virus growth (29).

The lack of response to antileukemic therapy is consistent with the experience of other investigators (5,6,10,12), who report a 1.5–4.5-month median survival in such patients once leukemia has been diagnosed. Whether this reflects a different biology of the disease, or resistance produced by prior exposure to chemotherapy, is unknown. The occurrence of a preleukemic phase in our patient, manifested by at least 3 months of asymptomatic monocytosis, is reminiscent of the pancytopenic periods noted prior to the onset of secondary malignancies in other reports (5,10,12,13). This may indicate an oligoleukemic syndrome which has been associated with a poor response to therapy (30).

The transient reduction in marrow infiltrate seen in our patient with *cis*-dichlorodiammineplatinum(II) treatment is interesting. This drug has been tested in acute leukemia and was found to have marginal activity, with four of 38 patients showing > 50% hematologic improvement and only one complete remission (31).

The CyVADIC regimen has been used in > 400 patients at our institution and in the Southwest Oncology Group since 1973. During this time there has been, to our knowledge, no other patient with sarcoma who has developed leukemia, although a formal analysis has not been performed. This incidence would seem to be quite low. The occurrence of secondary malignancies in patients with sarcoma, however, is limited, due to the poor survival of this group. In two large studies of long-term postchemotherapy survivors, leukemia developed 17–91 months after the primary neoplasm was found (5,6). Our patient's leukemia occurred at the lower extreme of this range. In view of the coincident progression of her sarcoma, she probably never would have achieved a 41-month survival, which is the median time to second malignancy development (5,6).

The past decade has seen the development of effective chemotherapeutic regimens for various forms of neoplasia. With the occurrence of prolonged survival in these patients, any late complications will become more evident. A constant vigilance, utilizing prospective long-term followup studies in cancer patients who have received antineoplastic therapy, will determine the true incidence of secondary malignancies.

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Evaluation of Single-Dose Vincristine, Actinomycin D, and Cyclophosphamide in Childhood Solid Tumors [1,2]

Daniel M. Green [3,4,*]

SUMMARY

Bolus chemotherapy with vincristine, actinomycin D, and cyclophosphamide was administered to 31 patients with solid tumors. Among 12 patients with measurable disease who had not received prior treatment, a complete response was achieved in one with Ewing's sarcoma and a partial response was achieved in another with rhabdomyosarcoma. The dose-limiting toxicity was granulocytopenia.

[Cancer Treat Rep 62:1517–1520, 1978]

Actinomycin D and cyclophosphamide have traditionally been administered to children with solid tumors using a daily schedule (1–3). Experimental data suggest that intermittent administration of these drugs at single, maximum tolerated doses might be more effective (4).[5] Recent reports have shown that actinomycin D can be administered safely using this schedule (5,6). In addition, using this schedule, actinomycin D was shown to be active in childhood lymphoblastic leukemia (6). Using a daily schedule, this drug had previously been shown to have no activity in this disease (7).

The present study examined the toxicity and activity of vincristine, actinomycin D, and cyclophosphamide (VAC) administered in combination, using an intermittent, bolus schedule, to children with solid tumors.

PATIENTS AND METHODS

Thirty-one patients with solid tumors were treated. The characteristics of the 21 patients with measurable disease are shown in tables 1 and 2. An additional ten patients without measurable disease were treated. Of these ten patients, six had received prior chemotherapy and radiation therapy and four had received no prior chemotherapy.

Chemotherapy consisted of vincristine (2 mg/m^2, maximum dose 2.0 mg), actinomycin D (1.0 mg/m^2), and cyclophosphamide (600 mg/m^2). Previously untreated patients received vincristine weekly during the first 6 weeks of therapy. Patients who were receiving or had just completed radiation therapy received reduced doses of actinomycin D (0.75 mg/m^2) and cyclophosphamide (450 mg/m^2). The doses of actinomycin D and cyclophosphamide were escalated, as shown in table 3, if the granulocyte count on Day 15 following the administration of chemotherapy exceeded 1200/mm^3. Thrombocytopenia was not a criterion for dosage reduction. Chemotherapy was administered every 21 days. Liver function tests and a urinalysis were obtained prior to the administration of chemotherapy.

Informed, written consent was obtained from the parents of all patients prior to the initiation of treatment.

The criteria for response were: *complete response* (CR)—disappearance of all palpable and/or radiographically demonstrable disease and absence of

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[3] Department of Pediatric Oncology, Sidney Farber Cancer Institute; Division of Hematology and Oncology, Children's Hospital Medical Center; and Department of Pediatrics, Harvard Medical School, Boston, Mass.

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[5] Skipper HE. Response of Ridgway osteogenic sarcoma (ROS) at different stages to a variety of drugs given according to different schedules. Birmingham, Southern Research Institute, 1977.

[*] *Reprint requests to:* Daniel M. Green, MD, Department of Pediatrics, Roswell Park Memorial Institute, 666 Elm St, Buffalo, NY 14263.

TABLE 1.—Previously untreated patients with measurable disease

Histology	Primary site	Metastases	Surgery	Radiation therapy	Response
Embryonal rhabdomyosarcoma	Right arm	No	Biopsy	No	NR
Embryonal rhabdomyosarcoma	Right thigh	No	Biopsy	No	NR
Alveolar rhabdomyosarcoma	Left chest wall	Lung	Biopsy	Yes (see text)	PR (8 mos)
Undifferentiated sarcoma	Left humerus	Lung	Biopsy	No	NR
Ewing's sarcoma	Left ilium	Right 11th rib	Biopsy	No	NR
Ewing's sarcoma	Left ilium	Lung	Biopsy	No	CR (15+ mos)
Ewing's sarcoma	Left pubic ramus	Skull	Biopsy	Yes	NR*
Ewing's sarcoma	Left 1st, 2nd, and 3rd ribs	Multiple bones	Biopsy	No	NR
Ewing's sarcoma	Right ilium	Multiple bones, bone marrow	Biopsy	No	NR
Dysgerminoma	Midbrain	Mediastinum	Biopsy	Yes	NR
Teratocarcinoma	Left ovary	Diffuse peritoneal	Partial excision	No	NR
Malignant schwannoma	Left chest	No	Biopsy	Yes	NR

* Did not receive cyclophosphamide.

TABLE 2.—Patients with measurable disease—prior chemotherapy

Histology	Previous chemotherapy*	Response
Hemangiosarcoma	VAC, adriamycin	NR
Ewing's sarcoma	VAC	NR
Undifferentiated sarcoma	VAC, adriamycin-DTIC, VM-26	NR
	VAC, adriamycin-DTIC	NR
Rhabdomyosarcoma	VAC, adriamycin-DTIC	NR
	VAC	NR
Hepatocellular carcinoma	Adriamycin	NR
	Adriamycin	NR
Diffuse histiocytic lymphoma	Vincristine, prednisone, and adriamycin	NR

* VAC (ref 9); adriamycin-DTIC (ref 13); VM-26 (ref 14).

symptoms suggestive of persistent or progressive disease; *partial response* (PR)— > 50% decrease in the product of the perpendiculars of one measurable lesion, absence of progression of any other measurable lesion, and absence of symptoms suggestive of progressive disease; and *no response* (NR)—progression of symptoms suggestive of disease activity or progression of any measurable lesion.

RESULTS

Twelve patients with measurable disease who had received no previous therapy were evaluated; two had objective responses. The responses, by tumor type, are shown in table 1. Of the five patients with Ewing's sarcoma, one who failed to respond never received cyclophosphamide. The patient with rhabdomyosarcoma who achieved a PR, as shown in table 1, achieved her response prior to the administration of radiation therapy. No responses were observed in the nine patients with measurable disease who had received prior chemotherapy.

Four patients treated with surgery and/or radiation therapy who received no previous chemotherapy had no evaluable disease when entered in the study. The histology and disease-free interval for these patients are: Sertoli-Leydig cell tumor (ovary) (13+ months), leiomyosarcoma (4+ months), rhabdomyosarcoma (7+ months), and undifferentiated sarcoma (6+ months).

Six patients were treated who had received prior radiation therapy and chemotherapy, but had no measurable disease when entered in the study. Five patients received this therapy after receiving combination therapy with adriamycin-cyclophosphamide (8) and one patient received VAC (9) and radiation therapy. One of these patients presented with Ewing's sarcoma of the humerus and ulna. Chemotherapy was electively discontinued 24 months after diagnosis, and this patient remains free of disease at 25 months. The duration of response from entry in the study of the remaining four patients with Ewing's sarcoma after therapy with VAC or adriamycin-cyclophosphamide was 3, 3,

TABLE 3.—Hematologic toxicity

			No. of episodes of—	
Dose*	Granulocyte nadir (cells/mm^3)	Monocytes (cells/mm^3)†	Fever and granulocytopenia	Severe thrombocytopenia (< 40,000 platelets/mm^3)
< 1.0/600	Mean, 1214 Range, 90–3036	294 0–1152	2	2
1.0/600	Mean, 1597 Range, 268–4290	622 0–1482	1	0
1.25/750	Mean, 979 Range, 26–3696	341 72–1120	1	1
1.50/900	Mean, 627 Range, 0–1599	775 0–1620	3	1

*Actinomycin D (mg/m^2)/cyclophosphamide (mg/m^2).
†On the day of the granulocyte nadir (Day 15).

4, and 13 months (three patients presented with hematogenous metastases and one with a pelvic primary). The sixth patient had a hemangiosarcoma and he had relapsed on VAC. This patient received whole-lung radiation therapy followed by chemotherapy with adriamycin-cyclophosphamide. Progressive pulmonary disease was documented 7 months after entry in the study.

Nausea and vomiting was universal. It was poorly controlled with antiemetics and generally persisted for 24–48 hours following chemotherapy.

The major toxicity was hematologic. As shown in table 3, myelosuppression was dose-related. Cbc counts, including platelet, wbc, and differential counts, were obtained from most patients 8 and 15 days following administration of chemotherapy. The platelet nadir generally occurred on Day 8, and the granulocyte nadir occurred on Day 15 following the administration of chemotherapy. Hospitalization for fever and granulocytopenia was required after 4% of the courses of chemotherapy (seven of 183). None of these hospitalizations was associated with severe infection (bacteremia or pneumonia). The next course of chemotherapy was delayed up to 7 days following some of these episodes to allow bone marrow recovery. Thrombocytopenia was the dose-limiting toxicity after 2% of the courses of chemotherapy (four of 183). Three of these episodes occurred shortly after the administration of radiation therapy. Although these episodes required platelet transfusion as a supportive measure, chemotherapy was administered on schedule at the same dose rate as the previous course of therapy.

Other toxic effects included severe stomatitis after 2% of the courses (four of 183) and obstipation after 1% (two of 183). Hemorrhagic cystitis occurred in 13% (four of 31) of the patients. Of the four patients with cystitis, one had previously documented cyclophosphamide-related cystitis, one received inadequate hydration, one had asymptomatic hematuria during a period of drug-induced thrombocytopenia (previous bladder radiation therapy), and one had previous bladder irradiation and had been adequately hydrated following administration of chemotherapy. Dysuria, without hematuria, occurred in an additional patient. A persistent papular skin rash was noted by one patient throughout the period of chemotherapy. This resembled previously described eruptions related to actinomycin D (10). No abnormalities of liver function were observed.

A number of the patients entered in the study had previously received adriamycin. Cardiomyopathy developed in only one patient who had received combination chemotherapy with adriamycin (cumulative dose, 450 mg/m^2), cyclophosphamide, and vincristine, had no direct myocardial irradiation, and had echocardiographic evidence of severe myocardial dysfunction prior to discontinuation of adriamycin. Following the development of overt congestive heart failure, the patient was treated with single-dose actinomycin D, mechlorethamine, digoxin, and diuretics with improvement of his clinical and echocardiographic abnormalities. No patient who had not previously been exposed to adriamycin developed evidence of cardiac dysfunction.

DISCUSSION

This study was undertaken to evaluate the activity and toxicity of combination chemotherapy with VAC administered on an intermittent single-dose schedule.

The literature contains very few reports of the activity of this combination of agents administered using a divided-dose schedule without the concurrent use of radiation therapy. In the Intergroup Rhabdomyosarcoma Study (IRS), the CR rate was 28% for group 3 patients (seven of 25) and 23% for group 4 patients (six of 26) prior to radiation therapy (11). PR rates prior to radiation therapy were not reported.

Patients with unresectable rhabdomyosarcoma of an extremity have a poor prognosis. In the IRS, the overall response rate was 64% for patients with group 3 rhabdomyosarcoma of an extremity to combined modality treatment (12), while the overall response rate was 82% for all patients with group 3 disease to combined modality treatment (2). The data published by the IRS do not allow evaluation of the response rate of patients with rhabdomyosarcoma of an extremity to chemotherapy only. There are no published data discussing the response rate of Ewing's sarcoma to combination chemotherapy with VAC using an intermittent, divided-dose schedule.

The number of patients included in the present study is too small to allow definite comparisons of response rates to be made. Objective responses occurred in one of three patients with rhabdomyosarcoma of the trunk or an extremity and in one of five patients with metastatic Ewing's sarcoma.

The toxicity of this combination of drugs, administered on an intermittent, single-dose schedule, has been manageable. The incidence of stomatitis, hospital admission for fever and granulocytopenia, and severe thrombocytopenia has been low. No significant infections (pneumonia or bacteremia) have occurred, and no other life-threatening complications have been observed. The only comparable published data are those of the IRS. Severe toxicity occurred in 58.5% and life-threatening toxicity occurred in 9.9% of group 3 and 4 patients treated with VAC (2).

The present data suggest that combination chemotherapy with VAC is well-tolerated. In addition, they suggest that single-dose therapy, using vincristine (2 mg/m^2, maximum dose 2.0 mg), actinomycin D (1.25 mg/m^2), and cyclophosphamide (750 mg/m^2) administered every 3 weeks will produce responses in previously untreated patients who are not receiving concurrent radiation therapy.

CONCLUSION

One hundred and eighty-three courses of intermittent, bolus chemotherapy consisting of VAC have been administered to 31 children with a variety of solid tumors. Toxicity has been reversible and manageable, with the most significant toxicity being granulocytopenia. Responses were observed in previously untreated patients with rhabdomyosarcoma and Ewing's sarcoma.

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ADDENDUM

Since this manuscript was submitted, one additional previously untreated patient with stage III embryonal rhabdomyosarcoma of the prostate was entered in the study. He achieved a CR and remains in the study at 4+ months. He has not received radiation therapy. All disease-free patients remain disease-free as of October 1978.

Treatment of Advanced Colorectal Cancer With Methyl-CCNU Plus 5-Day 5-Fluorouracil Infusion[1]

Robert C. Kane,* Michael R. Cashdollar, and Albert M. Bernath[2]

SUMMARY

Thirty-six patients with advanced colorectal carcinoma who had not received prior chemotherapy and had tumor sites measurable or evaluable for response received methyl-CCNU at a dose of 175 mg/m^2 orally on Day 1 plus 5-fluorouracil at a dose of 30 mg/kg/24 hours by continuous iv infusion for 120 hours (Days 1–5). Doses were chosen to approach the maximum which could be administered in combination. The cycle was repeated 6 weeks later. Objective partial responses occurred in six patients (17%), with five responses apparent after the first cycle and one additional response after the second cycle. The response duration ranged from 1.5 to 18+ months (median, 5 months). Dose-limiting toxic effects included mucositis in 18 patients (50%) and dermatitis in nine patients (25%), while leukopenia ($<$ 4000 cells/mm^3) and thrombocytopenia ($<$ 100,000 platelets/mm^3) were observed on at least one occasion during therapy in 52% and 46% of patients, respectively. 5-Fluorouracil administration by infusion avoided overlapping myelosuppression and allowed a higher total dose to be given with methyl-CCNU. However, the response to the combination did not exceed the results anticipated for the use of either drug alone.

[Cancer Treat Rep 62:1521–1525, 1978]

In the absence of data to establish optimal drug dose (based on pharmacokinetics, tumor cell growth kinetics, and host tolerance), we attempted to administer a maximal dose of methyl-CCNU and 5-fluorouracil (5-FU) in combination, based on an arbitrarily chosen acceptable incidence and severity of toxicity, to patients with advanced and metastatic colorectal cancer. While continuous 5-FU infusion has not been proven to result in increased response rates compared to intermittent injection, myelosuppression caused by 5-FU can be circumvented by continuous drug infusion, apparently by maintaining blood levels below a myelotoxic range. Seifert et al (1) explored dose-time relationships for infusions of 5-FU and found that 30 mg/kg/day over a 5-day period was usually tolerated satisfactorily. Myelosuppression was uncommon, but mucositis and rash were common and represented the dose-limiting toxicity. For these reasons, we chose to administer methyl-CCNU in the full dose recommended for single-agent treatment (175 mg/m^2 every 6 weeks) with a continuous 5-FU infusion of 30 mg/kg/day for 5 days every 6 weeks to patients with symptomatic advanced colorectal carcinoma.

METHODS

All patients referred to the oncology service for therapy for unresectable and/or metastatic colorectal cancer were considered eligible if they fulfilled the following criteria:

(*a*) Unresectable and/or metastatic colorectal cancer, histologically proven
(*b*) Measurable or clearly evaluable tumor for determination of response
(*c*) No prior chemotherapy
(*d*) Cbc counts within the normal range
(*e*) Expected survival of > 6 weeks
(*f*) Ambulatory performance status
(*g*) Informed consent given

Pretreatment evaluation included measurement of tumor, cbc and platelet counts, SMA-12 profile, carcinoembryonic antigen (CEA) (Roche), chest

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[1] Received June 9, 1978; accepted Aug 16, 1978.

[2] Department of Hematology-Oncology, Geisinger Medical Center, Danville, Pa.

**Reprint requests to:* Robert C. Kane, MD, Department of Hematology-Oncology, Geisinger Medical Center, Danville, Pa 17821.

TABLE 1.—Drug schedule

Drug and dose	Week		
	1	6	12
Methyl-CCNU 175 mg/m^2	Day 1	Day 43	
5-FU 30 mg/kg/day	Day 1 → 5	Day 43 → 47	

roentgenogram, liver scan, and other tests as needed for assessment of effects of treatment. Blood cell counts were repeated every 2 weeks. Tumor measurements, CEA titers, and pertinent roentgenograms or scans were obtained at least every 6 weeks.

Performance criteria were based on the Eastern Cooperative Oncology Group scale, and the criteria employed to determine response and progression, based on changes in measured tumors, were those of Moertel et al (2). All patients who received the first day of chemotherapy were considered evaluable. Survival time was calculated from the first day of chemotherapy.

Drug Schedule

Methyl-CCNU at a dose of 175 mg/m^2 (to the nearest 10 mg) was given on Day 1, and a continuous iv infusion of 5-FU at a dose of 30 mg/kg/day was initiated simultaneously and continued for 120 hours (5 days). Most infusions were maintained by use of a continuous infusion pump. This schedule was repeated on Day 43, 6 weeks after the start of the first cycle (table 1), with dose adjustments made for toxicity. The methyl-CCNU dose was reduced for myelosuppression and the 5-FU dose was reduced for severe mucositis or rash. In patients with progressive disease at 12 weeks, therapy was discontinued. Patients with stable or responding disease were offered an outpatient schedule of methyl-CCNU every 8 weeks plus 5-FU given by iv bolus every 2 weeks. However, four patients elected to continue to receive additional infusion cycles as above.

RESULTS

Of 44 patients who were invited to participate, eight patients declined. Thirty-six patients consented and were treated according to the protocol; none was lost to followup. All patients were considered evaluable. Pretreatment disease sites (some patients had more than one disease site) included 17 patients with local or residual tumor, 17 with liver metastasis, 14 with lung metastasis, and five with other abdominal, perineal, or pelvic tumors. Patient characteristics and results are indicated in table 2. Of the six responses, five occurred after one cycle at or before Day 43 (Week 6) of therapy. The other response occurred after two cycles (12 weeks). Four of six responders were women, but the difference in response rate between men and women was not statistically significant. Sixteen patients had progressive disease after two cycles. Tumor sites were unchanged (stable) in ten patients after two cycles of treatment. Seven of these patients progressed by 18 weeks and the other three progressed by Week 24. All six objective responders also had stabilization or improvement in disease-associated symptoms such as weight loss, jaundice, pain, etc. Two responding patients and two with stable disease at 12 weeks received additional infusion cycles of therapy. Other responding patients and those with stable disease were treated with outpatient regimens of methyl-CCNU and 5-FU as described

TABLE 2.—Summary of results in 36 patients with advanced colorectal cancer treated with methyl-CCNU and 5-FU infusion

No. of patients	36
Men	23
Women	13
Age in yrs	
Mean	59
Range	31–73
No. of patients with—	
Performance status	
1	23
2	13
Response	
Complete	0
Partial	6
Sites of response	
Lung	3
Liver	1
Abdomen	2
Disease progression	
Before 12 wks	4
At 12 wks	16
At 18 wks	7
At 24 wks	3
Duration of responses in mos	1.5, 2.5, 4+, 5, 8, 18+
% of calculated amount of drug administered	
Cycle 1	
Methyl-CCNU	99.7
5-FU	99.7
Cycle 2	
Methyl-CCNU	90
5-FU	95

TABLE 3.—Correlation of serial CEA titers (pretreatment, 6, and 12 wks) with clinical status

		No. of patients with—	
	No. of responders	Progressive disease at 12 wks	Progressive disease at 18–24 wks
Decreasing titer	5	1	2
Stable titer	0	3	3
Increasing titer	0	12	4
Total available for analysis	5	16	9

above, with doses adjusted as tolerated to obtain moderate reversible toxicity.

The duration of response was modest (median, 5 months), with two patients still responding at 4+ and 18+ months. The median survival is 9+ months for responding patients and 6 months for nonresponders.

CEA titers were measured before treatment and every 6 weeks thereafter. Pretreatment CEA titers were > 100 ng/ml in 14 patients and > 20 ng/ml in eight others. All patients were evaluated for response on the basis of clinical examination. Serial CEA titers were not used in the determination of response. However, the change in serial CEA titers correlated closely with the clinical assessment (table 3). Serial CEA titers were available in five of the six responding patients. Each of these five patients showed a serial decrease by ≥ 50% in the titer. Of 16 patients with progressive disease at 12 weeks, 12 had rising serial titers, three had stable (< 20% change) titers, and one showed a small decline (12.2–8.4–7.9 ng/ml). In nine patients whose objective disease status was stable at 12 weeks and who had serial CEA determinations, two showed decreasing titers and three had stable titers. Thus, serial CEA titers correlated highly with clinical assessment (chi-square = 22.5, $P < 0.01$) but were not infallible indicators of response status.

Toxicity

5-FU infusion in this dose range has previously been associated with little myelosuppression but has caused nonmyelosuppressive toxicity including rash and mucositis. Mucositis requiring a restriction of oral intake was experienced by 18 patients (50%); it usually lasted 4–6 days and occurred between Day 4 and Day 8 of treatment (table 4). No patients required iv management for this complication. A maculopapular, violaceous skin rash occurred in nine patients, seven of whom also had concurrent mucositis. A skin biopsy in one patient was interpreted to show a nonspecific polymorphous infiltrate. No neurologic toxicity from 5-FU was observed.

TABLE 4.—Toxicity from therapy in 36 patients

Toxic effect	% of patients
Mucositis	50
Rash	25
Platelet count—	
< 100,000/mm^3	46
< 50,000/mm^3	23
Leukocyte count—	
< 4000/mm^3	52
< 2000/mm^3	11
Bleeding	6
Infection	3

Myelosuppression followed the time course anticipated for methyl-CCNU therapy. Fifty-two percent of the patients had leukopenia with < 4000 cells/mm^3 on at least one occasion and 11% had a value of < 2000 cells/mm^3 on at least one occasion. Forty-six percent of patients had a platelet count < 100,000/mm^3 and one half of this group had at least one platelet count recorded at < 50,000/mm^3 during therapy. Ten percent of the patients' blood cell counts were not fully recovered by Week 6 for Cycle 2 but in only one patient was there a delay (1 week) in initiating the second cycle. No significant decline in hemoglobin level was observed during the 12-week period. One patient experienced severe mucositis, rash, and myelosuppression followed by sepsis and death occurring 3 weeks after therapy was started.

DISCUSSION

The intent of this program was to determine the response of patients with advanced colorectal cancer to methyl-CCNU and 5-FU when administered in maximally tolerated doses in combination. 5-FU

TABLE 5.—Protocol designs and treatment results in studies employing 5-FU infusion for colorectal cancer

Reference	Regimen	Response* (%)
Seifert et al (1)	5-FU, 12 mg/kg iv bolus × 5 days q4wks	8/36(22)
	vs	
	5-FU, 30 mg/kg/day infusion × 5 days q4wks	15/34(44)
Moertel et al (5)	5-FU, 13.5 mg/kg iv bolus × 5 days	9/74(12)
	vs	
	5-FU, 20 mg/kg iv 2-hr infusion × 5 days, or 5-FU, 25 mg/kg iv 2-hr infusion × 5 days	9/75(12)
Vaughn et al (6)	5-FU, 25 mg/kg/day infusion × 5 days q4wks, plus mitomycin C, 20 mg/m^2 iv bolus q8wks	? (68)
	vs	
	5-FU, 25 mg/kg/day infusion × 5 days q4wks, plus methyl-CCNU, 150 mg/m^2 orally q8wks	? (55)
Einhorn et al (7)	5-FU, 20 mg/kg/day infusion × 5 days q4wks, plus methyl-CCNU, 150 mg/m^2 orally on Day 1 q4wks	7/24(29)
Buroker et al (8)	5-FU, 25 mg/kg/day infusion × 4 days q4wks, plus mitomycin C, 20 mg/m^2 iv q8wks	34/124(19)
	vs	
	5-FU, 25 mg/kg/day infusion × 4 days q4wks, plus methyl-CCNU, 175 mg/m^2 orally q8wks	19/111(17)

* No. of patients responding/No. evaluated.

was given by infusion to obviate its myelosuppression and thus permit methyl-CCNU to be given in the full single-agent dose. Myers et al (3) have reported data in animals indicating a differential sensitivity to 5-FU between some tumor tissues and normal marrow and gastrointestinal epithelium. Methods of exploiting this possible differential sensitivity to increase the response rate to 5-FU without increasing toxicity might include continuous infusion, kinetically scheduled intermittent boluses, or infusion of higher doses of 5-FU with some form of rescue technique to abort toxicity. However, clinical trials to date do not show important differences in response rate with different schedules of administration.

Interest in 5-FU infusion has continued since the report by Lemon (4) of diminished toxicity without reduction in response rate by 8-hour infusions daily for 5–7 days. Several representative trials of 5-FU infusion in which sufficient data are available for analysis are presented in table 5. As usual, differences in the treatment schedule appear modest in comparison with the differences in results reported. Moertel et al (5) reported an equivalent 12% response rate when bolus administration was compared to a 2-hour infusion, each given daily for 5 days. The report of Seifert et al (1) suggested a favorable result in comparison with bolus administration, but differences in patient characteristics were noted to exist between the two groups. Randomization was not specified in the study of Vaughn et al (6) and the number of patients in each group was not given. Einhorn et al (7) reported minimal toxicity with moderately lower doses than we used, but the median duration of response (6 months) was equivalent to that of our series. The largest series to date comparing combination therapies including 5-FU infusion was by Buroker et al (8); they employed a 4-day infusion and attained a response rate (17%) identical to that of our series.

In the absence of pharmacokinetic modeling, we chose an infusion schedule calculated to produce definite but reversible toxicity and one which approximated the maximally tolerated doses of these two drugs. The calculated amounts of drug were administered according to the treatment plan, and 27 (75%) of the 36 patients experienced at least one dose-limiting effect (mucositis, rash, wbc count < 4000/mm^3, or platelet count < 100,000/mm^3) on at least one occasion. Unfortunately, although the clinically tolerated limits of this schedule were reached, no improvement in response rate or duration was achieved by this schedule beyond that expected for either of these drugs used alone.

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Phase I Clinical Study of Quelamycin [1]

A. Brugarolas,* N. Pachon, M. Gosalvez, A. Perez Llanderal, A. J. Lacave, J. M. Buesa, and M. Gracia Marco [2]

SUMMARY

A phase I clinical study was done with quelamycin, a recently synthesized triferric derivative of adriamycin. Twenty-one good-risk patients were studied: 19 patients with non-small cell carcinoma of the lung and two patients with metastatic sarcoma. Acute toxicity occurred in all patients and consisted of high fever, flushing, hypertension, generalized body aches, tremors, and confusion, which lasted 3–6 hours. Potentially dangerous cardiotoxicity occurred in eight patients who had previous minor rhythm disturbances, and was characterized by tachycardia, atrial extrasystoles, atrial fibrillation, and branch block which lasted 6–14 hours. The dose-limiting hematologic toxicity was found to occur at 125 mg/m^2 iv single-dose. Objective responses were observed in three of 19 patients with lung cancer and in one patient with metastatic osteogenic sarcoma resistant to adriamycin therapy. In conclusion, quelamycin is a new derivative of adriamycin with potential interest. However, the acute generalized toxicity and the immediate cardiotoxicity found in the presently used schedule are excessive. Further studies directed to suppress these side effects are in progress.

[Cancer Treat Rep 62:1527–1534, 1978]

Triferric doxorubicin (quelamycin; NSC-267703) is a new derivative of adriamycin which has been claimed to have similar antitumor activity with less cardiotoxicity (1,2). According to the studies of Gosalvez et al (3,4), adriamycin inhibited the activity of the heart Na-K-dependent adenosine triphosphatase, and this effect was abolished in the presence of calcium. It was postulated that adriamycin-induced cardiotoxicity might be mediated through this enzyme's inhibition, and therefore chelation of adriamycin might result in less cardiotoxicity. According to this hypothesis, quelamycin was synthesized with the objective of modifying or suppressing cardiotoxicity.

Preliminary reports appeared to confirm this hypothesis in the rat heart model, where less cardiac function impairment was found with quelamycin as compared to adriamycin (2). However, recent results suggest that acute and chronic cardiotoxicity might still be present (5).[3] Young et al (5) reported the development of adriamycin-like cumulative cardiotoxicity in rabbits given high-dose quelamycin. Although these studies have cast some doubt on the postulated absence of cardiotoxicity of quelamycin, the precise relevance of the model to man is not well-understood.

Preclinical studies identified pharmacologic differences between quelamycin and adriamycin (6). Quelamycin underwent less extensive metabolism than adriamycin and was relatively unaffected by aldo-ketoreductase and glycosidase. Some organ distribution differences were observed as well (6). Quelamycin crossed the blood-brain barrier in rabbits and was absorbed orally in dogs (2).

Regarding the antitumor effect of quelamycin,

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[2] Hospital General de Asturias, Oviedo (*A. Brugarolas, N. Pachon, A. Perez Llanderal, A. J. Lacave, J. M. Buesa,* and *M. Gracia Marco*), and Clinica Puerta de Hierro (*M. Gosalvez*), Madrid, Spain.

**Reprint requests to:* A. Brugarolas, MD, Hospital General de Asturias, c. J Claveria s.n., Oviedo, Spain.

[3] Pinedo H. Personal communication.

limited available data suggested activity comparable to that of adriamycin. In vitro DNA synthesis inhibition was similar for the two drugs (2,6). Comparable antitumor activity was found in L1210 leukemia, Walker 256 carcinosarcoma, Ehrlich ascites tumor, and P388 leukemia (2). Animal toxicology data are limited to mice. In this species, the LD50 was 45 mg/kg, three times the LD50 of adriamycin.

Preliminary toxicology studies in man done in Madrid reported a lack of acute and cumulative cardiotoxicity and confirmed the antitumor activity (7). Dose-limiting toxicity was hematologic, with patients showing great individual variability in tolerance. Several partial objective responses were detected among 16 treated patients: one anaplastic carcinoma of the lung, one adenocarcinoma of the lung, one gastric carcinoma, and one colon carcinoma. In that study, the recommended therapeutic dose was 150 mg/m^2 iv every 3 weeks.

In view of the relatively different pharmacology of the new drug and the reported antitumor activity, further testing was recommended. A conventional phase I clinical study was designed in Oviedo, and the results obtained are presented.

MATERIAL AND METHODS

Patients were selected according to the following criteria: (*a*) performance status > 40% on the Karnofsky scale, (*b*) pathology-proven advanced non-small cell carcinoma of the lung or metastatic soft tissue or osseous sarcoma, (*c*) measurable disease, and (*d*) major organ function within the normal limits. Patients with expected difficulties in followup were excluded. Patients with cardiorespiratory insufficiency, bone marrow impairment (wbc count < 4000/mm^3 and/or platelet count < 150,000/mm^3), renal disease (creatinine clearance < 80 ml/minute), hepatic failure, jaundice, psychosis, and central nervous system lesions were excluded. All patients were informed of the investigational character of the treatment and oral consent was obtained.

A complete history and physical examination, chest roentgenogram, electrocardiogram (ECG), urinalysis, cbc count, liver profile, and creatinine clearance were performed prior to treatment. Other studies were considered optional.

Quelamycin was supplied by Dr. M. Gosalvez in ampules containing a black lyophilized powder designed as a 25-mg equivalent of quelamycin to be reconstituted with 10 ml of water solvent. According to the information received, a 25-mg equivalent of quelamycin yields 25 mg of adriamycin upon dissociation of the iron ions. Reconstituted quelamycin was given iv at the rate of 5 mg (2 ml)/minute in a large vein of the arm.

The injection site was washed with a continuous iv running 5% dextrose in water solution during 2–3 hours (300–1000 ml).

Patients were maintained in the intensive care unit for 24 hours after the administration of quelamycin. Changes in temperature, blood pressure, heart rate, and ECG were recorded. All side effects were clearly defined. On the second day of treatment, patients were allowed to return to the hospital unit and careful observation was kept for 1 week. Major evaluation tests were repeated at weekly intervals and included physical examination, tumor measurement, performance status rating, blood cell counts, ECG, and serum chemistries. Blood cell counts were performed twice a week in hospital patients. A chest roentgenogram was repeated every other week.

The starting quelamycin dose was 75 mg/m^2 iv. Stepwise dose escalations were made with an increase of 25 mg/m^2 at each level until the dose-limiting toxicity was found. At least two patients were entered at each dose level. Every patient was maintained at the initial dose level unless toxicity occurred. No patient received two dose escalations. Patients were kept in the study until progression of disease, provided their life was not endangered. Treatments were repeated every 3 weeks unless toxicity was present.

Immediate acute toxicity was defined by all symptoms and changes noticed during the first 48 hours of treatment.

Hematologic toxicity was defined according to the following criteria: *none*—a wbc count of ≥ 4500/mm^3 and a platelet count of ≥ 300,000/mm^3; *mild*—a wbc count of 3000–4500/mm^3 and a platelet count of 200,000–300,000/mm^3; *moderate*—a wbc count of 2000–3000/mm^3 and a platelet count of 100,000–200,000/mm^3; *severe*—a wbc count of 1000–2000/mm^3 and a platelet count of 50,000–100,000/mm^3; and *life-threatening*—a wbc count of ≤ 1000/mm^3 and a platelet count of ≤ 50,000/mm^3.

Hepatic, renal, cardiac, mucocutaneous, and neurologic toxicities were investigated as well.

Response

Objective response was defined as a decrease of ≥ 50% in the size of the tumor as measured by the product of the two largest perpendicular diameters. Progression was defined as < 50% decrease in tumor size, mixed response, stable disease, and progressive growth of the tumor. Toxic death was defined as a death occurring during toxicity, including unexpected early death. Early death was defined as a death from progressive disease occurring before two cycles of treatment could be fully evaluated.

RESULTS

Twenty-one patients were studied. There were 19 males and two females whose ages ranged from 18 to 74 years (median age, 59). Nineteen patients had lung cancer: 11 with epidermoid carcinoma, one with adenocarcinoma, and seven with anaplastic large cell carcinoma. Six patients had stage III disease and 13 patients had stage IV disease. Metastatic sites included: liver, six patients; supraclavicular lymph nodes, six patients; bone, six patients; skin and soft tissues, three patients; and opposite lung, two patients. Eleven patients had received prior extensive chemotherapy (two to four drugs) and four had received two or more courses of adriamycin.

Another two patients had metastatic sarcoma of the lung. One patient was an 18-year-old male with metastatic osteosarcoma resistant to previous chemotherapy with adriamycin and DTIC. The other patient was a 51-year-old female with a rhabdomyosarcoma of the right thigh and pulmonary metastases who was treated with CYVADIC (cyclophosphamide, vincristine, adriamycin, and DTIC) and had massive progression of the disease.

Performance status according to the Karnofsky scale was 50% in three patients, 60%–70% in 15 patients, and ≥ 80% in three patients.

Twenty-one patients received a total of 51 courses of quelamycin at the time of this report. The number of cycles per patient ranged from one to five. Table 1 indicates the patient distribution according to dose level and number of cycles given.

Toxicity

Acute toxicity appeared 30 minutes to 4 hours after the administration of quelamycin (table 2). It appeared with facial flushing, sweating, thirst, chest pain, backache, bronchospasm, coughing and shortness of breath, tachycardia, hypertension, headache, high fever (up to 104°F), shaking tremors, and generalized malaise. It was not responsive to antihistaminics or corticosteroids, and ended spontaneously in 5–8 hours leaving no sequelae. Repeated administration of quelamycin was followed by a similar crisis, but occasionally it was better tolerated. Two patients had mild to moderate confusion and disorientation lasting 3 and 7 hours respectively.

Five patients had thrombosis of the injected vein. In three patients thromboses were extensive to the distal side of the injection. None of these patients had local tissue necrosis or extravasation of the drug during injection.

Complete alopecia was observed after the first cycle in all patients. Two patients had stomatitis 14 days after the first cycle.

Hematologic toxicity (table 3).—Dose-limiting toxicity was hematologic, with moderate and severe leukopenia and thrombocytopenia increasing in frequency with doses > 125 mg/m^2. The nadir occurred by Day 13 and appeared to occur earlier with the higher dose levels. Wbc and platelet counts returned to normal levels by Day 19. The median wbc nadir was related to the dose level given, as can be observed in table 4. No effect was observed on the hemoglobin. Cumulative toxicity could not be detected after three cycles.

Cardiotoxicity (table 5).—Acute cardiotoxicity could be studied in 21 patients. Eleven patients with a normal pretherapy ECG showed uncomplicated tachycardia with no side effects. Another two patients with a diagnosis of a possible previous myocardial infarction showed no significant changes other than tachycardia. On the contrary, patients with rhythm disturbances demonstrated potentially toxic complications. Five patients presented many atrial extrasystoles, and another four developed atrial fibrillation lasting 6–14 hours. One of these latter patients had a low output heart failure lasting 10 hours. The heart rate for the group of

TABLE 1.—Patient distribution by dose and No. of treatments

Dose level (mg/m²)	No. of patients	No. of cycles
75	2	2, 5+
100	2	1, 3
125	6	1+, 2, 2+, 3+, 3+, 4+
150	6	2, 2, 2+, 2+, 3+, 4+
175	5	1, 2, 2+, 2+, 3+

TABLE 2.—General toxicity of quelamycin: effects of the 1st course (21 patients)

	No. of patients with toxicity				
Type	None	Mild	Moderate	Total No.	%
Acute	1	4	16	20	95
Nausea and vomiting	7	9	5	14	66
Sympathomimetic reaction	3	7	11	18	85
Fever	2	3	16	19	90
Shaking tremor	4	11	6	17	80
Confusion and disorientation	19	0	2	2	9
Vein thrombosis				4	19
Alopecia				21	100
Stomatitis				2	9

TABLE 3.—Hematologic toxicity of quelamycin

Dose (mg/m²)	No. of patients treated	No. of evaluable courses/ total No. given	No. of courses with—									
			Wbc count (× 10^3/mm³)					Platelet count (× 10^5/mm³)				
			Normal	3–4.5	2–3	1–2	0–1	Normal	2–3.5	1–2	0.5–1	0–0.5
75	2	5/6	1	4	0	0	0	1	3	1	0	0
100	2	5/6	2	3	0	0	0	2	3	0	0	0
125	6	12/15	3	4	2	3	0	4	5	2	1	0
150	6	12/16	3	2	3	2	2	5	3	2	1	1
175	5	6/8	0	1	0	2	3	0	2	1	1	2

TABLE 4.—Wbc count data

Dose (mg/m²)	Median wbc count (× 10^3/mm³)		Wbc count nadir in days	
	Before treatment	At nadir	Median	Range
75	6	5.6	14	7–16
100	7.4	4.5	13	8–16
125	6.7	3.4	14	8–17
150	9.6	2.5	12	7–20
175	9.8	1.4	9	4–15

patients with rhythm disturbances was higher than for the normal ECG patients. Overall, there was no clear relationship between quelamycin dose, heart rate, and appearance of cardiotoxicity. Rapid digitalization was tried in three patients without improvement.

No liver, renal, or other organ toxicity could be identified. No toxic deaths occurred in this group of patients.

Four patients had serum iron determinations made at various intervals after treatment. Baseline serum iron ranged from 46 to 81 μg/dl. Thirty minutes after quelamycin administration, serum iron values ranged from 228 to 260 μg/dl. One hour after quelamycin administration, serum iron values ranged from 307 to 350 μg/dl. During the following 4–8 hours there was a progressive fall in serum iron which approached the baseline levels 6–12

TABLE 5.—Acute cardiotoxicity of quelamycin

Patient No.	Baseline ECG*	Quelamycin dose (mg/m^2)	No. of courses	Median frequency before dose	Median frequency after dose	Complications*
1	Normal	75	2	105	110	None
2	Normal	75	5	100	105	None
3	Normal	100	1	90	150	None
4	Normal	100	3	90	110	None
5	Normal	125	1	90	130	None
6	Normal	125	2	80	100	None
7	Normal	150	2	90	110	None
8	Normal	150	4	100	115	None
9	Normal	175	1	85	120	None
10	Normal	175	2	90	150	None
11	Normal	175	2	80	120	None
12	Previous MI	125	2	95	110	None
13	Previous MI	175	2	80	130	None
14	SVT	125	4	130	180	AF, 12 hrs (× 2)
15	AES	125	3	80	110	Many AES
16	AES	125	3	90	130	Many AES
17	AES	150	2	80	110	LASDB + AES
18	AES	150	3	80	160	AF, 6 hrs (× 1)
19	PE (LGL)	150	2	80	170	AF + BB, 6 hrs
20	LASDB	150	2	80	170	AF + AES, 12 hrs and AF + LOHF, 8 hrs
21	LASDB	175	2	90	140	Many AES

*Previous MI = previous myocardial infarction; SVT = supraventricular tachycardia; AES = atrial extrasystoles; AF = atrial fibrillation; LASDB = left anterior superior division branch block; PE (LGL) = pre-excitation syndrome of Lown-Ganon-Levine; and LOHF = low output heart failure.

hours after quelamycin administration. Twenty-four hours after the injection of quelamycin, serum iron was similar to the baseline control. Urinary iron excretion was high during the first 3 hours after quelamycin administration.

Response

Three objective responses were observed among 19 patients with lung cancer (15%). The first responding patient was a 58-year-old male with an untreated stage IV large cell anaplastic carcinoma of the carina with left supraclavicular lymph node metastases proven by biopsy who received quelamycin at a dose of 125 mg/m^2. After two cycles the decrease in tumor size qualified for an objective response. The supraclavicular mass decreased from 5.5 × 4.6 to 2 × 1.5 cm. Response has been maintained after five cycles of treatment.

The second patient was a 48-year-old male with a peripheral lesion in the right upper lobe with bilateral supraclavicular lymph node metastases proven to be giant cell anaplastic carcinoma by biopsy. Objective response was detected 5 weeks after starting quelamycin. The quelamycin dose given was 150 mg/m^2. This patient has a $>$ 75% objective response after four cycles of treatment.

The third patient was a 71-year-old female with an untreated large cell anaplastic carcinoma of the right hilum with lower lobe atelectasis and right pleural effusion with a positive cytology. Quelamycin (175 mg/m^2) was given. Three weeks after the first course of treatment, the hilar mass and the pleural effusion had cleared. Response has been maintained after three courses of treatment.

Another objective partial response was observed in a patient with a metastatic osteosarcoma refractory to adriamycin, which was given first as adjuvant postamputation therapy and then, after the appearance of pulmonary metastases, was given in combination with DTIC and methotrexate, up to a total adriamycin dose of 500 mg/m^2. Objective response was observed 2 weeks after the administration of the first dose of quelamycin. A 75% decrease in tumor size was observed after two cycles.

DISCUSSION

Acute quelamycin toxicity was similar to the crisis which follows iv iron administration (8). Systemic toxicity from parenteral iron administration includes immediate reactions such as headache, muscle pain, flushing, tachycardia, sweating, nausea and vomiting, and bronchospasm, and a delayed reaction occurring 1–24 hours after its administration including dizziness, confusion, chills, fever, convulsive seizures, and generalized body aches. Ionized iron is more toxic and its administration is followed by dizziness, hemolysis, hypotension, collapse, and shock. Iron toxicity usually occurs with iron doses $>$ 25 mg.

Clinically, it appeared that quelamycin acute toxicity possibly represented iron toxicity, and the data obtained with the serial serum iron determinations suggested this mechanism. Considering that 1 mg of adriamycin binds to 0.26 mg of iron ion, the estimated total iron administration in a single course per patient ranged in this study from 19 to 45.5 mg/m^2, which is well within the expected toxic dose levels.

Several facts suggest that the acute toxicity was not an anaphylactic type of reaction: lack of improvement with antihistaminics and corticosteroids, lack of recall phenomenon in the subsequent courses, interval between drug administration, and appearance of symptoms and spontaneous improvement in a few hours. Nevertheless, careful drug testing should be done to rule out drug impurities or excessive unbound iron.

Another distressing toxic manifestation found in this study was acute cardiotoxicity. It appeared to be mainly limited to patients with rhythm disturbances and was potentially dangerous. It was not clear if cardiotoxicity was due to a direct effect on the myocardium or an effect upon the heart innervation system. From the experimental data obtained in the heart model by Gosalvez et al, adriamycin was found to have an ouabain-type effect on the heart, with two distinct actions: (*a*) a direct stimulation of the muscle fibers, giving a positive inotropic effect, and (*b*) an action on the autonomic nervous system, resulting in tachycardia (1,2). These two actions have been clearly defined for digitalis in the transplanted heart model, where the action on the autonomic nervous system has been suppressed (9). Gosalvez et al (2) have claimed that chelation of adriamycin with iron would only remove the effect upon the myocardial fibers, leaving intact the effect upon the autonomic nervous system.

The cardiotoxicity found in the present study was mainly defined by tachycardia, arrhythmia, hypertension, and hyperexcitability, and could reflect effects of quelamycin upon the autonomic nervous system. However, iron toxicity might have contributed to the cardiotoxic effect; its exact role remains unknown at the present time.

On the other hand, cumulative heart toxicity was not documented. According to a recent analysis by Von Hoff et al (10), adriamycin cardiomyopathy is a rare event, occurring in only about 6% of the patients treated above the total adriamycin dose of 550 mg/m^2, and influenced by various factors such as age and concomitant irradiation of the mediastinum. Therefore, it can be anticipated that only a large series could answer the question of reduced long-term cardiotoxicity for quelamycin. Furthermore, patients selected for phase I studies are usually not suitable for long-term treatment, and unless the drug moves into phase II and III trials, this important question will possibly never be answered.

Regarding the antitumor activity of quelamycin, objective responses were observed in lung cancer and sarcoma. Active doses ranged from 125 to 175 mg/m^2. The response rate found in bronchogenic carcinoma is probably consistent with the expected response rate of adriamycin (11). The objective response observed in an adriamycin-resistant osteogenic sarcoma suggests a lack of cross resistance, which is certainly a feature which needs further documentation.

In conclusion, the dose-limiting toxicity of quelamycin appeared to be hematologic and the recommended therapeutic dose for good-risk patients appeared to be 125 mg/m^2 iv every 3 weeks. Nevertheless, the finding of unacceptable acute iron toxicity and acute cardiotoxicity probably prevents its present use at large doses in clinical trials in man until major improvements are made. Continuing investigations of schedule modification, drug treatment of adverse side effects, drug purification, and formulation are in progress. In addition, the long-term side effects need documentation.

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In Vivo Characteristics of Resistance and Cross-Resistance of an Adriamycin-Resistant Subline of P388 Leukemia [1]

Randall K. Johnson,[2,3,*] Manik P. Chitnis,[2,4] Wallace M. Embrey,[2] and Ernestine B. Gregory [2]

SUMMARY

A subline of P388 leukemia resistant to adriamycin (P388/ADR) was developed by exposure to the drug in vivo. Resistance to adriamycin proved to be a stable characteristic of P388/ADR. There was no significant inhibition of nucleic acid synthesis in P388/ADR cells in vivo following a dose of 10 mg/kg of adriamycin in contrast to a prolonged and complete inhibition, particularly of DNA synthesis, observed in parental sensitive P388 leukemia cells. P388/ADR proved to be completely cross-resistant to a spectrum of anthracycline derivatives. Cross-resistance was observed to nonanthracycline DNA intercalating agents (with the exception of anthramycin), to agents which interfere with mitotic spindle function, and to antineoplastic inhibitors of protein biosynthesis (with the exception of bruceantin). P388/ADR was sensitive to antimetabolites and alkylating agents. Cross-resistance was also observed to several agents (ICRF-159, a terephthalanilide, taxol, lymphosarcin, bouvardin, and a crude extract of *Ervatamia hyneana*) whose mechanisms of action have not yet been clearly defined. This observation has proved useful in providing a lead for determination of mechanism of action of some of these drugs. The pattern of cross-resistance of a subline of P388 leukemia resistant to daunorubicin, though not studied extensively, appears to be similar to that of P388/ADR.

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The anthracycline antibiotics, daunorubicin and particularly adriamycin, are effective in the treatment of a number of human tumors (1,2). Although remissions of hematologic malignancies and regressions of solid tumors can be dramatic and clinically beneficial, responses are often of relatively short duration and resistance to subsequent therapy is observed (3). A number of investigators (4–8) have studied resistance to anthracycline antibiotics in murine tumors. Reciprocal cross-resistance between the anthracycline antibiotics, vinca alkaloids, and actinomycin D has been observed in experimental studies (9).

We developed sublines of P388 leukemia resistant to adriamycin or daunorubicin in order to (*a*) investigate the mechanism of resistance to these agents, (*b*) attempt to find anthracycline analogs which would not be cross-resistant with adriamycin and daunorubicin, and (*c*) more clearly delineate the pattern of cross-resistance of anthracycline-resistant cells to a spectrum of cytotoxic agents. We reported previously (10) that the P388 adriamycin-resistant subline was cross-resistant to daunorubicin and several other anthracycline analogs. In this paper we present in vivo data which show that nucleic acid synthesis in resistant cells is not markedly inhibited following administration of a therapeutic dose of adriamycin. In addition, the adriamycin-resistant cell line is cross-resistant to a broad spectrum of cytotoxic agents with dissimilar chemical structure and mechanism of action.

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[2] Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md.

[3] *Present address:* Arthur D. Little, Inc, Cambridge, Mass.

[4] *Present address:* Cancer Research Institute, Parel, Bombay, India.

[*] *Reprint requests to:* Dr. Randall K. Johnson, Arthur D. Little, Inc, Acorn Park, Cambridge, Mass 02140.

MATERIALS AND METHODS

P388 Leukemia Cell Lines

Sublines of P388 leukemia resistant to adriamycin (P388/ADR) and daunorubicin (P388/DAU) were developed by treating B6D2F$_1$ mice bearing ascitic tumors with adriamycin or daunorubicin over successive transplant generations as previously described (10). Complete resistance to maximally tolerated doses of the anthracyclines in vivo was evident in both sublines after 12 transplant generations of exposure to the drugs. The cross-resistance studies reported in this paper were initiated on the 14th transplant generation of P388/ADR and on the 27th transplant generation of P388/DAU. Resistance to the anthracyclines was stable, being evident 70 transplant generations after discontinuing exposure to the drugs. The sublines were passaged in anthracycline-treated mice for 80 transplant generations prior to discontinuing treatment. Similarly, cross-resistance or sensitivity to a number of the drugs reported in this study was a stable characteristic of the sublines, proving reproducible when the sublines had been transplanted as many as 100 generations between tests. The sensitive parental P388 leukemia (P388/S) was carried by weekly transplantation in untreated syngeneic DBA/2 or B6D2F$_1$ mice.

Evaluation of Therapeutic Response of Resistant Sublines to Antitumor Agents

For chemotherapy studies, 10^6 viable cells of the P388 cell lines were inoculated ip in groups of eight B6D2F$_1$ mice. In all experiments, P388/S was evaluated in parallel with either or both of the resistant sublines. Seven drugs plus either adriamycin (for P388/ADR) or daunorubicin (for P388/DAU) were evaluated in each experiment. The drugs were tested at four dose levels, each dose level being 0.6 of the next highest. The dose levels were selected based on screening data so that the highest dose level would be toxic. The treatment schedule used was intermittent treatment on Days 1, 5, and 9 for the majority of drugs. Other schedules were used when necessitated by schedule dependency of a particular drug. Drugs were administered ip dissolved in appropriate aqueous vehicles. The following drugs could not be formulated in solution and were administered as suspensions: chartreusin, 4′,4″-bis(tetrahydropyrimidinyl)terephthalanilide, taxol, nitidine, oncodazole, and the methanol extract of *Ervatamia hyneana*.

The results were evaluated by comparing the median survival time for drug-treated groups with that of an untreated control group consisting of 24 mice in each experiment.

Conclusions as to whether sensitivity or cross-resistance was evident to a given compound were based on evaluation of complete dose-response curves. When median survival times of mice bearing P388/S and P388/ADR overlapped at several dose levels, a compound was concluded to have retained its activity against P388/ADR even though a rather large difference in percent increase in lifespan (ILS) might be evident at the optimal dose. Complete resistance was defined as $\leq 25\%$ ILS in P388/ADR when significant activity was evident against P388/S. Some compounds, eg, bleomycin and 6-mercaptopurine, could not be evaluated for cross-resistance since they do not significantly prolong the lifespan of mice bearing P388/S. Partial cross-resistance was evident with a few drugs characterized by extensive ILSs of P388/S compared to marginal activity in mice bearing P388/ADR.

Effects of Adriamycin on Nucleic Acid Synthesis in P388/S and P388/ADR In Vivo

P388/S or P388/ADR was inoculated ip (10^6 viable cells) in large groups of B6D2F$_1$ mice. Six days later the mice were treated with a maximally tolerated dose of adriamycin (10 mg/kg, ip). At various intervals thereafter, groups of three mice were injected ip with 10 microcuries of (methyl-^{3}H)-thymidine (2 curies/mmol) and 1 microcurie of 2-^{14}C-uridine (6.4 millicuries/mmol), both purchased from the New England Nuclear Corp, Boston, Mass. After a 30-minute pulse period the animals were killed, ascitic tumor cells were collected, and the specific activity of DNA and RNA was determined as previously described (11). To determine the effects of adriamycin on thymidine labeling index, 20 microcuries of (methyl-^{3}H)-thymidine (20 curies/mmol) was injected ip to groups of three mice at various intervals after adriamycin administration. The animals were killed 30 minutes after administration of the labeled nucleoside and smears of ascitic fluid were made. Autoradiograms were made with Kodak AR–10 stripping plates (Eastman-Kodak, Rochester, NY). The emulsion was exposed for 7 days and the slides were stained with hematoxylin and eosin. The labeling index was determined by counting 1000 cells on each slide and counting a cell as labeled if more than five grains were present over the nucleus. The average grain count of P388/S cells was determined by counting the grains over the nuclei of 60 labeled cells.

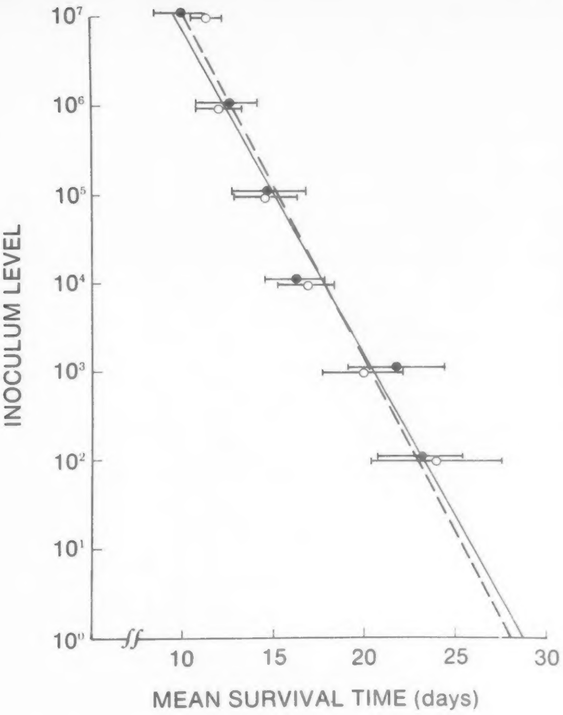

FIGURE 1.—Survival time of B6D2F$_1$ mice bearing ip P388/S or P388/ADR as a function of cell inoculum. P388/S = closed symbols, solid line; P388/ADR = open symbols, dashed line. Each symbol = the mean ± SD survival time of a group of 10 mice. The lines were drawn by regression analysis.

RESULTS

P388/ADR was virtually indistinguishable from the parental P388 leukemia with respect to its growth characteristics in untreated syngeneic B6D2F$_1$ mice as shown in figure 1. This titration comparison, performed with P388/ADR in the 120th serial transplantation passage, gave an estimated in vivo doubling time of 18.2 hours for P388/ADR and 19.4 hours for P388/S. Thus, an equivalent prolongation of lifespan of mice bearing the sensitive and resistant tumors represents equivalent cell-kill.

The adriamycin-resistant subline is completely resistant to the therapeutic effects of maximally tolerated doses of adriamycin in vivo as previously described (10). This therapeutic resistance was reflected biochemically in a lack of significant inhibition of the incorporation of labeled thymidine and uridine into the nucleic acids of P388/ADR cells following adriamycin treatment of mice bearing ascitic tumors (fig 2). The specific activity of DNA and RNA in P388/S cells fell rapidly following administration of adriamycin; incorporation of thymidine was completely inhibited from 6 to 72 hours following drug administration whereas uridine incorporation recovered progressively at 48 and 72 hours. The thymidine labeling index of P388/S cells decreased only slightly during the first 12 hours following adriamycin treatment (fig 3), although the mean grain count decreased by 4 hours in agreement with the reduced specific activity of DNA. By 24 hours, the labeling index of sensitive cells was essentially zero and labeled cells remained absent up to 96 hours after drug administration. The labeling index of P388/ADR cells was not decreased at any time following administration of adriamycin.

We previously reported (10) that P388/ADR was cross-resistant to the following anthracycline derivatives: daunorubicin, carminomycin, cinerubin A, nogalamycin, rubidazone, and adriamycin-14-octanoate. We have subsequently evaluated a broad spectrum of active anthracycline derivatives (table 1). P388/ADR was cross-resistant to all of the analogs tested. These compounds include adriamycin and daunorubicin derivatives modified in the sugar moiety (*N*-dimethyl, *N*-trimethyl, and *N*-dibenzyl analogs; an *N*-acylated derivative, AD-32; and the 4′-epimer of adriamycin), derivatives with modifications in the aglycone (4-demethoxy analogs and 5-iminodaunorubicin), adriamycin complexes with DNA or ferric ion, and a potential double intercalator in which two daunorubicin molecules are linked together in the 13-positions with a succinylhydrazone moiety. Resistance was also evident for naturally occurring and semisynthetic anthracycline antibiotics including: nogamycin and *O*-methylnogarol, semisynthetic analogs of nogalamycin; ϵ-pyrromycinone-containing antibiotics, pyrromycin, musettamycin, marcellomycin, and cinerubin B; and aclacinomycin A. The greatest ILS of mice bearing P388/ADR by any of these anthracyclines was 31% with AD-32. However, adriamycin in the same experiment also gave 31% ILS in P388/ADR. The optimal response to adriamycin, run in parallel with the analogs in each experiment, is also shown in table 1 for comparison.

The adriamycin-resistant subline was cross-resistant not only to DNA intercalating agents with the anthracycline structure but to a variety of DNA intercalating agents of great diversity in chemical structure (table 2). The cross-resistant DNA intercalating agents included: antibiotics, actinomycin D (12), azetomycin II (13), mithramycin (14), and chartreusin (15); alkaloids, ellipticine (16), nitidine (17),

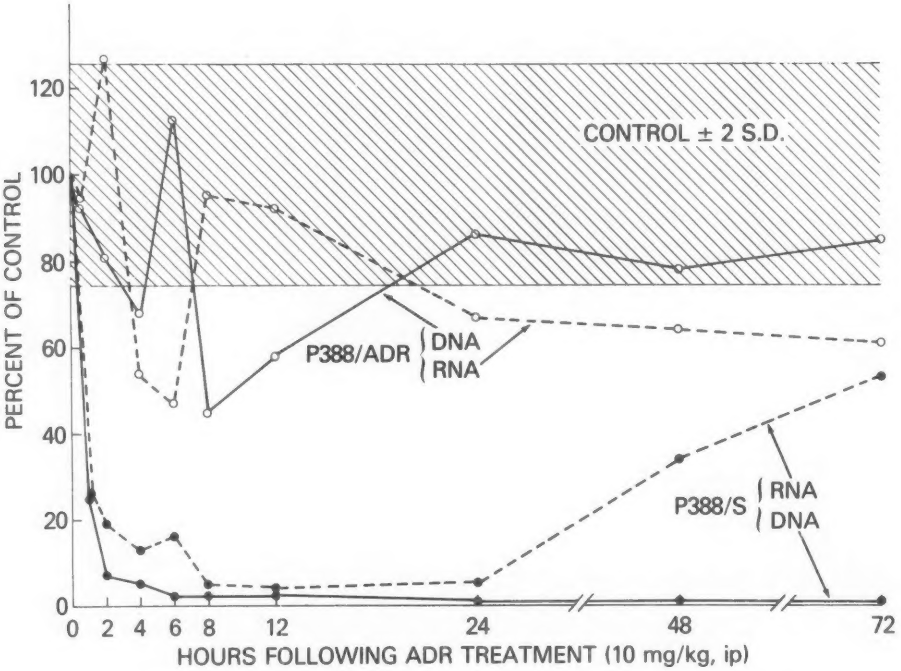

FIGURE 2.—Time course of inhibition of thymidine incorporation into DNA and uridine incorporation into RNA in P388/S and P388/ADR cells in vivo following a single dose of adriamycin (10 mg/kg ip). Each point = the average of duplicate determinations on 3 mice.

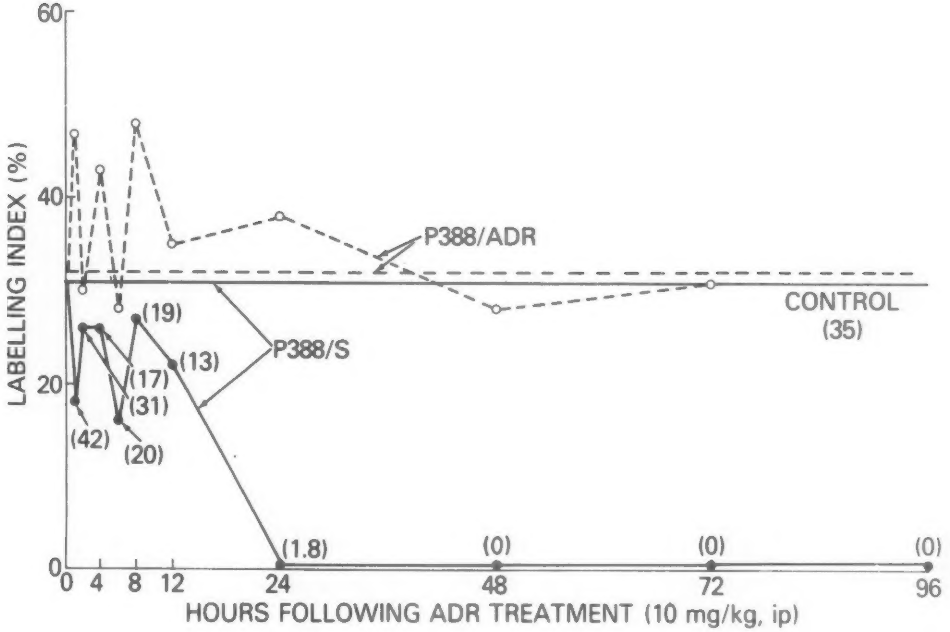

FIGURE 3.—Thymidine labeling index of P388/S and P388/ADR cells in vivo following a single dose of adriamycin (10 mg/kg ip). Each point = the average labeling index for 3 mice. Values in parentheses = mean grain counts for labeled nuclei of P388/S cells.

TABLE 1.—Effects of anthracycline derivatives on the lifespan of mice bearing P388/S or P388/ADR*

			P388/S		P388/ADR		Optimal response (%ILS) to adriamycin in—	
NSC No.	Drug	Dose range (mg/kg/injection ip)	Optimal dose (mg/kg)	%ILS	Optimal dose (mg/kg)	%ILS	P388/S	P388/ ADR
	Adriamycin derivatives							
261045	*N*-dimethyladriamycin	1.44–6.67	2.40	91 (1/8)	2.40	14	123 (1/8)	10
249335	*N*-trimethyladriamycin	8.64–40.0	14.4	40	14.4	0	80	0
256942	4′-*epi*-Adriamycin	2.16–10.0	3.60	70	3.60	0	80	0
256438	4-Demethoxyadriamycin	0.216–1.00	0.600	70	0.600	0	80	0
246131	*N*-(trifluoroacetyl)-adriamycin-14-valerate (AD-32)	13.0–60.0†	36.0	255 (2/8)	36.0	31	109†	31
169534	Adriamycin-DNA complex	1.73–8.00†	4.80	186	1.73	4	109†	31
267703	Adriamycin-Fe^{+++} chelate (1:3, quelamycin)	6.48–30.0	18.0	86	18.0	9	114	9
	Daunorubicin derivatives							
258812	*N*-dimethyldaunorubicin	3.46–16.0	9.60	85	9.60	18	80	0
256439	4-Demethoxydaunorubicin	0.324–1.50	0.540	104	0.900	18	221	32
254681	5-Iminodaunorubicin	3.46–16.0	5.76	77	9.60	18	109	17
266210	Bis(daunorubicin)-13-succinylhydrazone	8.64–40.0	24.0	70	14.4	5	80	0
268242	*N*-dibenzyldaunorubicin	7.78–60.0	21.6	73	21.6	7	109 (1/8)	0
	Other naturally occurring or semisynthetic anthracyclines							
265450	Nogamycin	4.32–20.0	7.20	77	7.20	0	123	18
269148	*O*-methylnogarol	18.0–83.3	30.0	162 (2/8)	30.0	14	221	32
267229	Pyrromycin	4.32–20.0	20.0	55	20.0	12	109	17
219941	Musettamycin	3.46–16.0	5.76	109	3.46	8	109	17
265211	Marcellomycin	1.08–5.00	1.80	86	3.00	21	123	18
18335	Cinerubin B	0.864–4.00	2.40	62	2.40	27	125	18
208734	Aclacinomycin A	3.24–15.0	9.00	95	3.24	21	123	18

*P388/S and P388/ADR were evaluated in the same experiment with each agent listed. Adriamycin was included as a control at 4 dose levels in each experiment. In 22 experiments the optimal %ILS for adriamycin varied from 80% to 230% (mean ± SD; 140% ± 37% ILS) for P388/S and from 0 to 32% (mean ± SD; 17% ± 9% ILS) for P388/ADR. Fractions in parentheses = tumor-free survivors/total on Day 60. Drugs were administered on Days 1, 5, and 9 unless indicated by a †.

†AD-32, adriamycin-DNA complex, and adriamycin in this experiment were administered on Days 1–4.

TABLE 2.—Effects of nonanthracycline DNA intercalating agents on the lifespan of mice bearing P388/S or P388/ADR*

			P388/S		P388/ADR	
NSC No.	Drug	Dose range (mg/kg/injection ip)	Optimal dose (mg/kg)	%ILS	Optimal dose (mg/kg)	%ILS
3053	Actinomycin D	0.108–0.500	0.300	145 (1/8)	0.300	18
244393	Azetomycin II	0.216–1.00	0.600	164	0.360	8
24559	Mithramycin	0.324–1.50†	0.540	64	0.540	0
71795	Ellipticine	21.6–100	36.0	118	36.0	4
5159	Chartreusin	43.2–200	120	80	120	0
146397	Nitidine	54.0–250	54.0	69	54.0	8
154890	Coralyne	86.4–400	144	100	86.4	0
142982	Hycanthone	17.3–80.0†	80.0	91	48.0	4
249992	AMSA	5.40–25.0	9.00	100 (1/8)	9.00	14
196473	Anthracenedione	13.0–60.0	21.6	205 (1/8)	21.6	9
106408	Anthramycin	0.151–0.700	0.252	110	0.252	100

**See* footnote * for table 1.

†Schedules of administration for mithramycin and hycanthone were Days 1–5 and 1–9 respectively.

TABLE 3.—Effects of mitotic spindle poisons on the lifespan of mice bearing P388/S or P388/ADR*

			P388/S		P388/ADR	
NSC No.	Drug	Dose range (mg/kg/injection ip)	Optimal dose (mg/kg)	%ILS	Optimal dose (mg/kg)	%ILS
67574	Vincristine	0.648–3.00	1.08	133	1.08	14
49842	Vinblastine	0.648–3.00	1.80	141	1.08	17
245467	Vindesine	0.648–3.00	1.80	125 (2/8)	1.08	14
122819	VM-26	2.88–13.3	4.80	> 400 (4/8)	4.80	32
141540	VP-16-213	18.0–83.3	18.0	295 (3/8)	30.0	17
153858	Maytansine	0.054–0.250	0.150	115 (1/8)	0.250	15
238159	Oncodazole	21.6–100	60.0	180	36.0	59

*See footnote * for table 1.

TABLE 4.—Effects of inhibitors of protein biosynthesis on the lifespan of mice bearing P388/S or P388/ADR*

			P388/S		P388/ADR	
NSC No.	Drug	Dose range (mg/kg/injection ip)	Optimal dose (mg/kg)	%ILS	Optimal dose (mg/kg)	%ILS
33669	Emetine	7.78–36.0	21.6	121 (1/8)	13.0	12
129414	(±)-2,3-Dehydroemetine	10.80–50.0	30.0	50	30.0	8
131548	*O*-methyltubulosine	10.8–50.0	18.0	68	18.0	8
39147	Streptovitacin A	0.173–0.800†	0.480	73	0.173	5
141633	Homoharringtonine	0.720–3.33	2.00	65	2.00	8
52947	Pactamycin	5.18–24.0	8.64	58	8.64	0
141537	Anguidine	4.32–20.0	12.0	64	7.20	31
	Anguidine	1.08–5.00†	3.00	91	1.80	52
138780	T2-toxin	1.08–5.00	3.00	77	3.00	29
	T2-toxin	0.540–2.50†	1.50	155	0.540	24
165563	Bruceantin	0.432–2.00‡	1.20	100	1.20	50
	Bruceantin	0.389–1.20†	0.389	60	0.648	41

*See footnote * for table 1.
†These drugs were administered on Days 1–9.
‡Bruceantin was administered on Days 1–4 in this experiment.

and coralyne (18); and synthetic agents, hycanthone (19), AMSA (20), and an anthracenedione derivative (21). The only intercalating agent to which P388/ADR was sensitive was anthramycin. This agent binds to DNA with evidence of the formation of covalent linkage (22). In this respect, anthramycin may be considered a DNA-binding alkylating agent rather than purely an intercalating agent.

P388/ADR was also cross-resistant to a number of cytotoxic agents which interfere with mitotic spindle function (table 3) including vinca alkaloids (23), epipodophyllotoxin derivatives (24,25), and the ansa macrolide, maytansine (26). Partial resistance was observed with the insoluble, small molecular weight synthetic agent, oncodazole, which has been shown to cause metaphase arrest (27).

One further group of cytotoxic drugs to which P388/ADR proved to be cross-resistant was inhibitors of protein biosynthesis (table 4). Cross-resistance was observed to inhibitors of the elongation step of ribosomal protein synthesis including benzoquinolizidine alkaloids, emetine (28), (±)-2,3-dehydroemetine (29), and *O*-methyltubulosine (30), and a glutarimide antibiotic, streptovitacin A (31). Cross-resistance was also evident to a number of inhibitors of the initiation phase of eukaryotic ribosomal protein synthesis including homoharringtonine (32), pactamycin (33), and the trichothecene mycotoxins, anguidine and T2-toxin (34,35). The plant product, bruceantin, which has been shown to be an inhibitor of initiation (36), retained considerable activity against the adriamycin-resistant subline. With the trichothecene mycotoxins, a small difference in structure, the presence (T2 toxin) or absence (anguidine) of an isovaleryl ester in the 8α position, influenced the degree of resistance. Anguidine showed partial cross-resistance in several experiments, whereas T2 toxin, more effective than anguidine against P388/S, was consistently less active against P388/ADR.

TABLE 5.—Effects of antimetabolites on the lifespan of mice bearing P388/S or P388/ADR*

			P388/S		P388/ADR	
NSC No.	Drug	Dose range (mg/kg/injection ip)	Optimal dose (mg/kg)	%ILS	Optimal dose (mg/kg)	%ILS
740	Methotrexate	10.8–50.0	18.0	128	18.0	88
19893	5-Fluorouracil	21.6–100	60.0	122	60.0	92
102816	5-Azacytidine	6.48–30.0	6.48	114	6.48	109 (1/8)
63878	Cytosine arabinoside	504–2330	1400	177 (2/8)	1400	146 (2/8)
143467	DL-Alanosine	173–800	480	95	288	58
118994	Inosine dialdehyde	108–500	300	136	300	100
4728	2-Aminothiadiazole	173–800	173	82	173	100
163501	ACIA	21.6–100	21.6	62	60.0	42

*See footnote * for table 1.

TABLE 6.—Effects of alkylating agents on the lifespan of mice bearing P388/S or P388/ADR*

			P388/S		P388/ADR	
NSC No.	Drug	Dose range (mg/kg/injection ip)	Optimal dose (mg/kg)	%ILS	Optimal dose (mg/kg)	%ILS
26271	Cyclophosphamide	108–500†	180	389 (3/8)	108	> 400 (8/8)
8806	Melphalan	3.60–16.7†	10.0	272 (3/8)	10.0	> 400 (6/8)
119875	*cis*-Dichlorodiammineplatinum(II)	1.08–5.00	1.08	118	1.80	> 400 (5/8)
762	Mechlorethamine	0.432–2.00	0.432	100 (2/8)	0.720	86 (1/8)
135758	Piperazinedione	1.44–6.67	4.00	164 (2/8)	4.00	117
26980	Mitomycin C	1.08–5.00	3.00	136	3.00	> 400 (6/8)
409962	BCNU	13.0–60.0†	36.0	> 400 (6/7)	36.0	> 400 (6/8)
178248	Chlorozotocin	6.48–30.0	10.8	385 (3/8)	10.8	> 400 (5/8)
101536	Emetine mustard	4.32–20.0	12.0	42	20.0	39 (1/8)

*See footnote * for table 1.
†These drugs were administered on Day 1 only.

P388/ADR was not cross-resistant to any of a number of antimetabolites (table 5). The resistant line proved to be sensitive to an antifol, methotrexate; pyrimidine antagonists, 5-fluorouracil, 5-azacytidine, and cytosine arabinoside; inhibitors of de novo purine biosynthesis, DL-alanosine (37), inosine dialdehyde (38), and 2-aminothiadiazole (39); and a glutamine antagonist, L-[αS,$5S$]-α-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid (ACIA) (40).

Alkylating agents were also highly active against P388/ADR (table 6). Sensitivity was observed to bis(2-chloroethyl)amine substituted compounds such as cyclophosphamide, melphalan, and mechlorethamine, as well as to more complex alkylating agents such as mitomycin C and the nitrosoureas. It is interesting to note that emetine mustard, with substitution of the isoquinoline nitrogen of emetine with a bis(2-chloroethyl)aminoethyl moiety, had low activity against P388/S and equivalent activity against P388/ADR. This is in contrast to the complete resistance of P388/ADR to emetine.

A number of agents with activity against P388/S but with mechanisms of action which either have not been clearly elucidated or differ from those of compounds in tables 2–6 were evaluated against P388/ADR (table 7). Camptothecin and neocarzinostatin are thought to act by fragmenting the DNA of sensitive cells (41,42). Both of these agents were active against P388/ADR. Activity in P388/ADR was also observed with indicine-*N*-oxide. This agent belongs to the class of pyrrolizidine alkaloids, compounds with alkylating activity (43). P388/ADR was partially cross-resistant to a terephthalanilide derivative and to Cain's quinolinium. Complete cross-resistance was evident to the plant products, taxol and bouvardin, and to a crude extract from the Indian plant, *Ervatamia hyneana*. Resistance was also evident with the polyene antibiotic, lymphosarcin, and the synthetic agent, ICRF-159.

Limited in vivo evaluation of P388/DAU was carried out. This subline appeared to have a pattern of cross-resistance similar to P388/ADR (table 8).

TABLE 7.—Effects of antineoplastic agents with unknown mechanism of action on the lifespan of mice bearing P388/S or P388/ADR[*]

NSC No.	Drug	Dose range (mg/kg/injection ip)	P388/S		P388/ADR	
			Optimal dose (mg/kg)	%ILS	Optimal dose (mg/kg)	%ILS
100880	Camptothecin	18.0–83.3	30.0	100	30.0	108
157365	Neocarzinostatin	1.08–5.00	1.80	155	1.80	108
132319	Indicine-*N*-oxide	173–800†	288	46	288	50 (1/8)
57153	4′,4″-Bis(tetrahydropyrimidinyl)-terephthalanilide	10.8–50.0	18.0	105	18.0	32
176319	Cain's quinolinium	4.32–20.0†	7.20	159	7.20	58
125793	Taxol	25.9–120	25.9	82	43.2	4
208642	Lymphosarcin	2.88–13.3	8.00	69	4.80	8
129943	ICRF-159	108–500	180	164	108	3
259968	Bouvardin	4.32–20.0	7.20	110	7.20	0
B668619	Methanol extract of *Ervatamia hyneana*	108–500	300	55	500	12

[*] *See* footnote * for table 1.

† These drugs were administered on Days 1–9.

TABLE 8.—Effects of various antineoplastic agents on the lifespan of mice bearing P388/S or P388/DAU[*]

NSC No.	Drug	Dose range (mg/kg/injection ip)	P388/S		P388/DAU		P388/ADR†
			Optimal dose (mg/kg)	%ILS	Optimal dose (mg/kg)	%ILS	
82151	Daunorubicin	1.73–8.00	1.73	120	1.73	12	R (ref 10)
123127	Adriamycin	1.73–8.00	2.88	210	2.88	8	R (ref 10)
18334	Cinerubin A	1.73–8.00	4.80	170	4.80	25	R (ref 10)
3053	Actinomycin D	0.108–0.500	0.180	225 (1/8)	0.180	17	R (table 2)
67574	Vincristine	0.432–2.00	0.720	195	0.432	8	R (table 3)
245467	Vindesine	0.648–3.00	1.80	125 (2/8)	1.08	0	R (table 3)
33669	Emetine	7.78–36.0	21.6	140	21.6	0	R (table 4)
52947	Pactamycin	5.18–24.0	8.64	58	5.18	0	R (table 4)
19893	5-Fluorouracil	21.6–100	60.0	75	60.0	92 (1/7)	S (table 5)

[*] *See* footnote * for table 1.

† Results of studies in P388/ADR: R = resistant, S = sensitive.

Cross-resistance was observed to the anthracyclines, adriamycin and cinerubin A, and to actinomycin D, vinca alkaloids, and protein synthesis inhibitors. P388/DAU was sensitive to 5-fluorouracil.

DISCUSSION

Studies in experimental tumor systems and in cultured cell lines have indicated an unusual pattern of cross-resistance between the anthracycline antibiotics, actinomycin D, and the vinca alkaloids (9). The study presented here indicates that this pattern of cross-resistance may be much broader, including a spectrum of cytotoxic agents which act by three disparate mechanisms of action, namely, binding to DNA, binding to tubulin, and inhibition of protein biosynthesis. We have also investigated the pattern of cross-resistance of a vincristine-resistant subline of P388 leukemia (P388/VCR) (unpublished observations). This subline was cross-resistant to many of the drugs to which the anthracycline-resistant sublines were also cross-resistant, including the protein synthesis inhibitors and a number of DNA-binding agents. There were, however, a number of differences between the patterns of cross-resistance of P388/ADR and P388/VCR. P388/VCR was cross-resistant to daunorubicin but not to adriamycin. In contrast to P388/ADR, P388/VCR was completely cross-resistant to anthramycin but retained sensitivity to ellipticine. These qualitative differences in the pattern of cross-resistance would suggest that the mechanisms of resistance in these two sublines are not identical. P388/VCR, like P388/ADR, proved sensitive to antimetabolites and alkylating agents.

The pattern of cross-resistance displayed by these sublines of P388 leukemia probably is not evident in clinical cancer (or quite possibly even in resistant sublines of other murine tumors) since there is no evidence of cross-resistance in humans treated with the anthracycline antibiotics and the vinca alkaloids. Nevertheless, P388/ADR can serve as a useful tool for antineoplastic drug development because the finding of resistance or sensitivity to an unknown agent can supply a valuable lead toward determination of its mechanism of action. The finding of cross-resistance for two of the drugs listed as agents with unknown mechanism of action in table 7 led us to further studies which defined their mechanisms of action. Taxol, a plant product with a unique and unusual structure (44), was shown to be a mitotic spindle poison using a cytomorphologic assay (45). Bouvardin, a cyclic hexapeptide plant product (46), was shown to be an inhibitor of protein synthesis (47). The finding of cross-resistance could suggest specific experiments to determine whether an unknown agent binds to DNA or tubulin or inhibits protein synthesis.

One of our original reasons for developing the anthracycline-resistant sublines was to search for unique anthracycline derivatives which would not be cross-resistant. As can be seen from table 1 and from previously reported studies (10), 25 anthracyclines were tested and cross-resistance was observed for P388/ADR to all of them. These included *N*-(trifluoroacetyl)-adriamycin-14-valerate and *O*-methylnogarol, two anthracycline derivatives with extensive antitumor activity but which have been shown to lack the interaction with DNA (48,49) that characterizes other anthracycline derivatives (50). It is possible that these agents work by some mechanism other than DNA intercalation since (*a*) P388/ADR is cross-resistant to many agents which do not intercalate into DNA, and (*b*) adriamycin has been shown to interfere with cellular respiration (51), produce free radicals (52,53), and affect the cell membrane (54). It is conceivable that one or more of these effects contribute to the cytotoxicity of adriamycin and that derivatives which do not intercalate into DNA may retain cytotoxic activity.

The majority of the anthracycline analogs tested, with the exception of AD-32 and the DNA complex of adriamycin, had activity similar to or less than that of adriamycin against P388/S as shown in table 1. Since these experiments were not designed to define relative effectiveness against the sensitive line, the apparent superior activity of AD-32 and the adriamycin-DNA complex (or the apparent inferiority of several other anthracyclines) observed in single experiments should not be construed as definitive. The variation in optimal response to adriamycin of P388/S (80%-230% ILS) in 22 experiments indicates the necessity of multiple direct comparison experiments for ranking of relative effectiveness in the sensitive cell line.

Chinese hamster ovary cell lines with resistance to actinomycin D induced by continuous exposure in vitro proved to be cross-resistant to a spectrum of cytotoxic agents with diverse mechanisms of action (55). The degree of resistance was proportional to the molecular weight of the various test compounds. The mechanism of resistance in these cell lines was subsequently shown to be due to altered cell membrane composition (56) which impedes the uptake of large molecular weight compounds. Cross-resistance in P388/ADR was related to the specific classes based on mechanism of action rather than to chemical nature or molecular weight. The best example of this is the complete resistance of P388/ADR to emetine as compared to the sensitivity of P388/ADR to emetine mustard. The substitution of the isoquinoline nitrogen of emetine with the alkylating moiety renders the derivative inactive as an inhibitor of protein synthesis (57) and results in an alkylating agent with a somewhat higher molecular weight than emetine. On the other hand, relatively small changes in molecular structure of the trichothecene mycotoxins with no apparent effect on mechanism of action (34,35) affect the degree of resistance of P388/ADR as shown in table 4. Although it could be argued that the compounds to which P388/ADR is cross-resistant are generally larger than those compounds to which it is sensitive, this is probably a reflection of the preponderance of natural products in the classes of DNA binders, tubulin binders, and protein synthesis inhibitors. Natural products tend to be larger and to have more complex structures than synthetic agents which comprise the classes of antimetabolites and alkylating agents. A number of relatively small molecules did show cross-resistance, ie, ICRF-159, streptovitacin A, ellipticine, and oncodazole. On the other hand, P388/ADR was sensitive to some large molecular weight antineoplastic agents such as neocarzinostatin and bruceantin.

We have studied at the biochemical level the mechanism of resistance of P388/ADR to adriamycin and daunorubicin (58), actinomycin D (59), and emetine (60). With adriamycin and daunorubicin there was somewhat decreased uptake of these drugs in P388/ADR and P388/DAU relative to P388/S. However, there was a much greater difference in the retention of the anthracycline antibiotics between the sensitive and resistant sublines. Adriamycin or daunorubicin that had been taken up by P388/ADR and P388/DAU was subject to a much more rapid efflux as compared to P388/S, and resistance was clearly related to this enhanced efflux rather than to decreased uptake (58). With actinomycin D (59) and emetine (60) there was no significant impediment to drug uptake but, as with the anthracycline antibiotics, these drugs were retained by the resistant lines for a much shorter time than by P388/S cells. The failure of adriamycin to inhibit DNA or RNA synthesis in P388/ADR cells in vivo as shown in figures 2 and 3 is probably a reflection of both the decreased uptake and the decreased retention of the drug by the resistant cells. Emetine, in contrast, inhibited protein and, subsequently, DNA synthesis in both P388/ADR and P388/S cells in vivo following administration of a therapeutic dose to tumor-bearing animals. The duration of inhibition was much shorter in P388/ADR cells (60), reflecting the decreased retention of this drug. Active outward transport of anthracycline antibiotics has been demonstrated in Ehrlich carcinoma cells (61), and this transport was shown to be enhanced in a daunorubicin-resistant subline of this tumor (62). The broad pattern of cross-resistance of P388/ADR would suggest the possibility of a common pathway in the cellular pharmacodynamics of DNA intercalators, tubulin binders, and protein synthesis inhibitors. Obviously the intracellular targets of these various agents are different. These studies would suggest the possibility that there may be a common efflux mechanism for these xenobiotics and that enhanced activity of this efflux mechanism can render tumor cells resistant to these agents. The mechanism of resistance of P388/ADR and P388/DAU appears to be identical (58,59), and this is reflected in a qualitatively similar pattern of cross-resistance. On the other hand, P388/VCR appears to be resistant by virtue of decreased drug uptake (63), and the pattern of cross-resistance of this subline is different from that of the anthracycline-resistant sublines, as discussed above. It may be that the diminished uptake evident with adriamycin and daunorubicin may also play a role in the resistance of P388/ADR to some of the agents studied in this report.

The sensitivity of P388/ADR to antimetabolites and alkylating agents remains to be explained. P388/ADR appears to be more sensitive to a number of alkylating agents (cyclophosphamide, melphalan, *cis*-dichlorodiammineplatinum(II), and mitomycin C) than P388/S. This apparent collateral sensitivity was reproducible in repeat experiments and was evident for the nitrosoureas as well as for the agents mentioned above. Although it is not apparent from the summarized data shown in table 6, BCNU and chlorozotocin were curative in P388/ADR at suboptimal doses which did not cure mice bearing P388/S. It is possible that antimetabolites and alkylating agents do not share the efflux transport mechanism which functions for the DNA intercalators, tubulin binders, and protein synthesis inhibitors. Alkylating agents may not require maintenance of intracellular drug levels for very long to produce lethal damage, whereas interference with DNA and tubulin function and inhibition of protein synthesis may have to be maintained for several minutes or hours before a cell is irreparably damaged. Antimetabolites (or their active anabolic products) must be maintained for a finite period in cells to cause cell death. However, these agents, as congeners of normal metabolites, may not be handled by the same transport mechanism(s) as the drugs to which P388/ADR is cross-resistant.

A generalized uptake or efflux transport mechanism for xenobiotics in tumor cells, if one exists, could be important in cancer chemotherapy and warrants further study. An effective inhibitor of efflux transport could potentially enhance the activity of cytotoxic agents and reverse resistance in cell lines like P388/ADR or P388/DAU.

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Comparative Effects of Citrovorum Factor and Carboxypeptidase G_1 on Cerebrospinal Fluid-Methotrexate Pharmacokinetics[1,2]

Herbert T. Abelson,* William Ensminger, Andre Rosowsky, and Jack Uren[3,4]

SUMMARY

A comparison between citrovorum factor (CF) and carboxypeptidase G_1 ($CPDG_1$) rescue with respect to cerebrospinal fluid (CSF)-methotrexate (MTX) disappearance was studied in a patient with recurrent medulloblastoma who had a ventriculoperitoneal shunt. $CPDG_1$ rescue resulted in a prolonged CSF-MTX half-life of 16.5–23 hours in comparison with CF rescue where the CSF-MTX half-life was 6.5–7.2 hours. There was a positive clinical response measured by loss of bone pain, increased physical activity, and almost complete clearing of CSF blast cells. $CPDG_1$ rescue after high-dose MTX may provide more intense and selective treatment for meningeal neoplasms.

[Cancer Treat Rep 62:1549–1552, 1978]

Methotrexate (MTX) is a phase-specific agent which exhibits no cytotoxicity against G_0 or resting cells (1). It is, therefore, important to maintain "threshold" MTX levels for as long as possible to optimize cell kill (2). This concept of concentration × time (C × T) has been exploited in designing better regimens using MTX to treat meningeal leukemia (3). In addition to meningeal leukemia, other meningeal tumors (4), as well as some parenchymal central nervous system (CNS) tumors (5,6), are responsive to MTX.

In order to maximize C × T with high-dose MTX for CNS therapy, we have begun to rescue some patients with the enzyme carboxypeptidase G_1 ($CPDG_1$) (7–10) rather than with citrovorum factor (CF). $CPDG_1$ is a large molecule (7) and should be excluded from the cerebrospinal fluid (CSF) by the blood-brain barrier. This exclusion should provide localized therapy since MTX would be hydrolyzed to a nontoxic product in the blood but not in the CSF, and correspondingly, the C × T for MTX in the CSF would be prolonged and unopposed by rescue.

This paper compares the effect of $CPDG_1$ and CF on the egress of MTX from the CSF.

MATERIALS AND METHODS

Patients

Patient 1 is a 13-year-old white male with recurrent medulloblastoma, first diagnosed when he was 8 years old and treated with surgical extirpation followed by 4000 rads of whole-brain irradiation, 900-rad boost to the posterior fossa, and 3800 rads to the neuraxis. Four years later, the tumor recurred in the posterior fossa and left frontal region and another 2900 rads was delivered to the whole brain. One year later, a computerized tomographic scan showed hydrocephalus without further tumor recurrence. Dexamethasone was given to the patient and a ventriculoperitoneal shunt was implanted. CSF at that time revealed sheets of medulloblastoma cells. In addition, the patient had diffuse

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[3] Department of Pediatrics and Biological Chemistry, Harvard Medical School; Division of Hematology-Oncology, Children's Hospital Medical Center; and the Sidney Farber Cancer Institute, Boston, Mass.

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**Reprint requests to:* Herbert T. Abelson, MD, Sidney Farber Cancer Institute, 44 Binney St, Boston, Mass 02115.

bone pain. Plain films and a bone scan showed diffuse disease throughout the skeleton.

Drug and Treatment Plan

MTX was obtained from the National Cancer Institute. $CPDG_1$ was obtained from Dr. Henry Blair at the Tufts-New England Medical Center. MTX was administered at a dose of 7.5 g/m^2 for 24 hours by continuous infusion with one tenth of the total dose given over the first hour and the remainder over 23 hours. At the end of the MTX infusion, CF was begun at 15 mg every 3 hours as previously described (11). For the courses rescued by $CPDG_1$, 50 IU/kg of $CPDG_1$ was given iv over 5 minutes at the end of the MTX and then every 12 hours for a total of four doses.

Clinical and Laboratory Studies

Cbc counts, serum creatinine, BUN, and liver function tests were obtained prior to each treatment and repeated daily during the MTX infusion and for 48 hours after rescue with either CF or $CPDG_1$. The creatinine clearance was > 80 ml/minute prior to each treatment course.

MTX levels were determined by radioimmunoassay (RIA) as previously described (12) after CF rescue and by high-pressure liquid chromatography (HPLC) (Waters) using a μBondapak CN column (3.9 mm internal diameter × 30 cm) following $CPDG_1$ rescue. Details of this procedure will be presented elsewhere.[5]

$CPDG_1$ activity was monitored by a spectrophotometric assay as previously described (7).

CSF cell morphology was monitored by cytocentrifuge.

RESULTS

MTX pharmacokinetics in the CSF were influenced by the type of rescue utilized. Figure 1 shows the disappearance of MTX from the CSF after three courses rescued by CF and after two courses rescued by $CPDG_1$. The first two courses of high-dose MTX were followed by CF and the half-lives for CSF-MTX were 6.5 and 7 hours, respectively. In an attempt to optimize C × T, the next two courses were followed by $CPDG_1$ with MTX half-lives in the CSF of 16.5 and 23 hours, respectively. The fifth course of therapy was followed again by CF because the patient had developed low-titer antibody to $CPDG_1$. The half-life of MTX on that course was 7.2 hours.

$CPDG_1$ was not detectable in any of the CSF specimens following $CPDG_1$ rescue. After the first two courses consisting of MTX-CF, the cell count in the CSF went from confluent sheets of blast cells to < 10 cells/mm^3 but the cytocentrifuge still showed some tumor clumps. After three additional courses, two with $CPDG_1$ and the last with CF rescue, the cell count was 0, but cytocentrifuge analysis continued to show a rare positive cell or tumor clump. Clinically, there was loss of headache and complete relief from bone pain. No evidence of leukoencephalopathy was apparent.

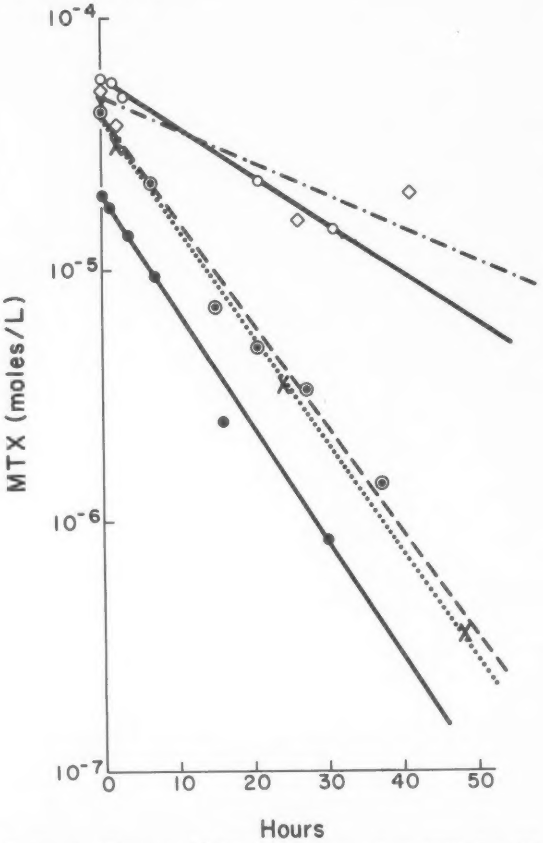

FIGURE 1.—Kinetics of MTX disappearance from CSF after CF or $CPDG_1$ rescue. At Time 0, the MTX infusion was ended and CF or $CPDG_1$ was given iv. The 0 points were obtained prior to beginning rescue. For the 3 courses rescued with CF, MTX levels were determined by RIA. For the two courses rescued with $CPDG_1$, MTX levels were determined by HPLC. The same MTX schedule and dose were given for each course. ●——— = Course 1, CF; × .... = course 2, CF; ○ ——— = course 3, $CPDG_1$; ◇ — · ——— = course 4, $CPDG_1$; and ⊙ - - - - - - - = course 5, CF.

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[5] Abelson HT, Ensminger WE, Rosowsky A, et al. Degradation of serum methotrexate with carboxypeptidase G_1: a possible approach to central nervous system neoplasia. Manuscript in preparation.

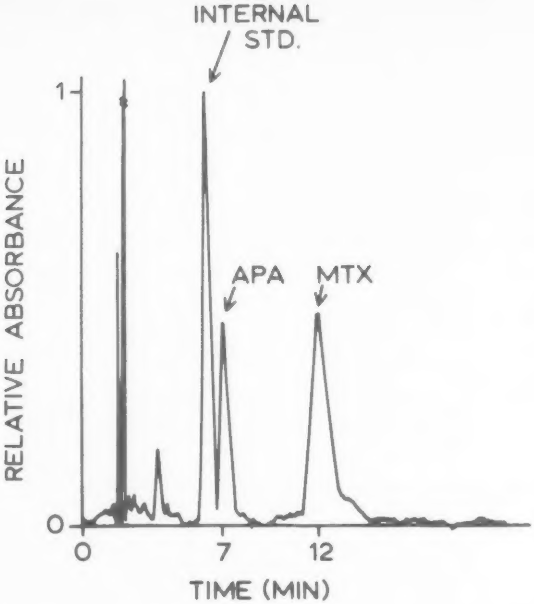

FIGURE 2.—Separation of MTX and APA by HPLC. MTX and APA were mixed in equimolar amounts (2.5×10^{-5} M) with human serum that had been previously deproteinized. 4-Dimethylaminobenzoic acid was added as an internal standard. The column was eluted with a buffer containing 0.005 M ammonium acetate and 0.008 M tetrabutylammonium hydroxide in 10% ethanol (adjusted to pH 7.6 with acetic acid). The flow rate was 2 ml/min.

Serum-MTX levels prior to rescue were determined by RIA and varied between 1.3×10^{-4} and 2.5×10^{-4} M for the five courses. These prerescue MTX values were confirmed by HPLC analysis. In addition, when HPLC was used to quantitate CSF-MTX levels after CF rescue, MTX levels and the resulting CSF-MTX half-lives were the same as those determined by RIA.

During the two courses following $CPDG_1$, the hydrolysis of serum MTX was followed by HPLC because the RIA cannot distinguish the cleavage product 4-amino-4-deoxy-N^{10}-methyl pteroic acid (APA) from MTX. HPLC separates MTX from APA, thus allowing us to monitor the precursor, MTX, and the cleavage product, APA. Figure 2 shows that the resolution of MTX and APA by HPLC is complete. The unidentified serum components which run with the solvent front do not interfere with the MTX and APA quantitation.

DISCUSSION

$CPDG_1$ offers a regional approach to systemic chemotherapy with MTX. $CPDG_1$ is excluded from the CSF, thereby providing more favorable C × T and, equally important, there is no exogenous rescue of the CSF such as would be provided by CF administration. Endogenous rescue of the CSF should also be less since $CPDG_1$ would be expected to cleave 5-methyltetrahydrofolate, the predominant form of folate in the serum.

We have shown for the first time that MTX disappearance from the CSF is significantly affected by the mode of rescue. With CF rescue, the half-life of about 7 hours is in close agreement with that reported for high-dose MTX followed by CF or for intraventricular administration without rescue (13,14). In contrast, the CSF-MTX half-life is more than doubled after $CPDG_1$ rescue. The mechanism for slower MTX clearance is not apparent. Changes in simple diffusion and bulk flow of CSF, alterations in choroid plexus physiology (15,16), and interactions between MTX and 5-methyltetrahydrofolate transport (17) are considerations. There is no information available as to whether $CPDG_1$ induces changes in CSF passive diffusion. There is, however, direct evidence in dogs and rabbits that MTX is partially cleared from CSF by a saturable transport process in the choroid plexus (16,18). Indirect evidence for a saturable transport mechanism is also present in man (19). The latter might then involve a countertransport phenomenon with administered folates facilitating transport of MTX out of CSF (17). Probenecid also prolongs CSF-MTX levels (18), but in contrast to $CPDG_1$, it will also affect renal tubular transport (20), thereby delaying serum-MTX excretion.

The patient presented in this paper has had a definite response to high-dose MTX as has been reported for other patients with recurrent medulloblastoma (5). The decrease in the number of tumor cells in the CSF and relief from bone pain began with the first two courses of high-dose MTX-CF and continued through the subsequent treatment. The unexpected development of enzyme-inactivating antibodies to $CPDG_1$ required the re-institution of CF rescue. The kinetics of MTX egress from CSF on that last course with CF were the same as the first two courses with CF, suggesting that the slower clearance after the $CPDG_1$ courses was not due to alterations in the "tightness" of the blood-brain barrier or to tumor-induced changes in CSF flow. The extrapolated half-life of CSF-MTX after $CPDG_1$ rescue indicates that "therapeutic" (13) levels $> 10^{-6}$ M will be present for > 100 hours. In contrast, the MTX-CF courses have CSF-MTX levels $> 10^{-6}$ M for < 50 hours, and in addition, there is added exogenous 5-methyltetrahydrofolate. Human glioblastomas have cell cycle times of approximately 2–3 days (21); however, little is known about recurrent medulloblastomas. Utilizing the 2–3-day estimate, unopposed 10^{-6} M MTX would be present for two cell cycles. The prolongation of C × T after $CPDG_1$ rescue has obvious advantage for meningeal disease, and it may also facilitate treatment of parenchymal brain lesions by diffusional transport from the CSF (22).

The results in this patient will need to be confirmed; however, they do suggest that $CPDG_1$ rescue may offer a therapeutic modality of great potential in the treatment of CNS neoplasia.

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Brief Reports
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Clinical Phase I-II Study of *cis*-Dichlorodiammineplatinum(II) Given by Continuous Iv Infusion[1,2]

Philip Salem, Stephen W. Hall,* Robert S. Benjamin, William K. Murphy, J. Taylor Wharton, and Gerald P. Bodey[3,4]

SUMMARY

To determine the efficacy and toxicity of *cis*-dichlorodiammineplatinum(II) (DDP) by a continuous iv infusion (CIVI) schedule, 34 patients with a variety of solid tumors were studied. All patients were refractory to prior chemotherapy and received a loading dose of 5 mg/m^2 of DDP iv followed by 20 mg/m^2 by CIVI daily for 5 days. In 25 evaluable patients, there were four (16%) complete or partial responders, nine (25%) with stable disease, and 12 (48%) with tumor progression. One complete and one partial remission were seen in two patients with disseminated basal cell carcinoma, with partial responses also seen in cervical and head and neck squamous cancer. Patients experienced renal damage (21%) and audiotoxicity (10%). Nausea and vomiting was severe in only 6%. DDP by CIVI appears to have comparable toxicity to DDP administered by other schedules; however, the diminished gastrointestinal toxicity makes the drug better tolerated by patients for whom the inconvenience of a 5-day hospitalization is less than that caused by the nausea and vomiting of rapid infusion programs.

[Cancer Treat Rep 62:1553–1555, 1978]

cis-Dichlorodiammineplatinum(II) (DDP) is clinically active against a wide spectrum of solid tumors, with best responses seen in testicular, ovarian, squamous head and neck, and bladder carcinomas (1–3). The drug has been most commonly given in a high single dose or by daily rapid infusion with mannitol or fluid-induced diuresis. Toxic manifestations of these schedules have been severe nausea and vomiting, mild to moderate myelosuppression, and dose-limiting nephrotoxicity (4,5). Although mannitol and fluid-induced diuresis have diminished the incidence of nephrotoxicity (6), renal impairment remains a major impediment to long-term or high-dose therapy. In an attempt to diminish the renal toxicity of DDP, we administered the drug by continuous iv infusion (CIVI). Also, in vitro studies have demonstrated greater cell-killing by prolonged exposure to low doses of DDP and suggest that CIVI would produce an enhanced antitumor effect of DDP (7,8).

PATIENTS AND METHODS

Thirty-four patients with a variety of recurrent or disseminated solid tumors refractory to prior treatment were studied (table 1). All patients gave written informed consent according to institutional policy before treatment. All patients had measurable tumor and adequate renal function (serum creatinine < 1.5 mg/100 ml).

Evaluation before therapy included quantitative measurements of evaluable tumor, cbc count, SMA-12 profile, urinalysis, creatinine clearance, chest roentgenogram, serum electrolytes, bone marrow aspiration and biopsy, and audiogram. Appropriate additional roentgenologic, isotopic, and laboratory studies were performed when indicated. Blood counts, BUN, and creatinine were repeated weekly. Creatinine clearances were repeated before each course of therapy. Audiograms and examinations used to quantitate changes in the tumor were repeated during every other course of treatment.

A complete response (CR) was defined as the complete disappearance of all tumor-related symp-

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[3] Department of Developmental Therapeutics and Gynecology, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

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**Reprint requests to:* Stephen W. Hall, MD, Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, Tex 77030.

TABLE 1.—Continuous infusion DDP: clinical response

	No. of patients		No. responding				Response duration (mos)		
Diagnosis	Entered	Evaluable	CR	PR	Stable disease	PD	CR	PR	Stable disease
Breast carcinoma	14	11			5	6			3,3,2,2,2
Squamous cell carcinoma									
Head and neck	7	4		1	2	1		3+	3,2
Cervix	1	1		1				3+	
Vulva	1	1				1			
Skin	1	0							
Basal cell carcinoma	2	2	1	1			4+	7+	
Melanoma	2	2				2			
Adenoid cystic carcinoma	1	1			1				3
Seminoma	1	1				1			
Thymoma	1	1			1				2
Embryonal cell carcinoma	1	0							
Renal carcinoma	2	1				1			
Total	34	25	1(4%)	3(13%)	9(36%)	12(48%)			

toms and apparent disease. A partial response (PR) was a ≥ 50% reduction in the sum of the products of the two greatest perpendicular diameters of all measurable lesions without the appearance of new lesions. Stable disease was no objective change in the evaluable disease parameters for at least 8 weeks. Progressive disease (PD) was defined as a 25% increase in tumor measurements or the appearance of any new lesions.

DDP was diluted with 100 ml of normal saline and a dose of 5 mg/m^2 was administered iv as a loading dose over 30 minutes. Immediately following the loading dose, 20 mg/m^2 of DDP in 1–1.5 liters of normal saline or 5% dextrose-normal saline was administered daily in three or four divided doses as a continuous infusion for 5 days. Subsequent doses and courses of treatment were given at 3–4-week intervals depending on preceding toxicity.

RESULTS

Antitumor Effect

The patients' diagnoses and responses to therapy are shown in table 1. In the 25 evaluable patients there were three PRs (12%), one CR (4%), nine stable diseases (36%), and 12 PDs (48%). The overall response rate was 15%. Best responses were one CR and one PR in two patients with basal cell carcinoma and two PRs (33%), two stable diseases (33%), and two PDs (33%) in six patients with squamous cancer of the head and neck or cervix. Patients with breast cancer comprised the greatest number of patients; there were five stable diseases (45%) and six PDs (55%) among 11 patients. Sites of CR included labia and groin lesions and sites of PR included clearing of bone marrow infiltration with basal cell carcinoma. PRs were also seen in patients with facial and neck recurrences of squamous cancer.

Toxicity

The toxic manifestations of DDP by CIVI are shown in table 2. Major clinical toxicity included nausea with or without vomiting which occurred in 94% of the patients but was severe in only 6%. Mild nausea and vomiting occurred in 60% of the patients, predominantly on the first day of treatment. This toxicity was perhaps related to the loading dose of DDP. In most patients antiemetics were not required after the first day and many patients did not require them at all.

Nephrotoxicity (serum creatinine > 2 mg/100 ml) occurred in 21% of the patients as manifested by a median BUN of 32 mg/100 ml (range, 23–45 mg/100 ml) and a median serum creatinine of 2.5 mg/100 ml (range, 2.0–3.7 mg/100 ml). The nephrotoxicity was not progressive, since most patients had improvement in renal function after DDP treatment was stopped.

Audiotoxicity occurred in 10% of the patients and was evidenced by complaints of tinnitus or documented decrements in hearing determined by serial audiograms. Tinnitus occurred on the last 1 or 2

TABLE 2.—Continuous infusion DDP: nonhematologic toxicity

	No. (%) of—	
	Evaluable patients (total = 33)	Evaluable courses (total = 61)
Gastrointestinal	31(94)	43(70)
Nausea only	5(15)	7(11)
Nausea + vomiting*		
Mild	15(45)	24(39)
Moderate	9(27)	11(18)
Severe	2(6)	2(3)
Diarrhea	2(6)	2(3)
Epigastric pain	2(6)	2(3)
Nephrotoxicity	7(21)	7(11)
Audiotoxicity	3(10)	3(5)
Headache	1(3)	1(2)
Phlebitis	1(3)	1(2)
Fever	1(3)	1(2)
Infection	2(6)	2(3)

*Mild = no antiemetics or only 1 antiemetic dose required for symptomatic improvement; moderate = >1 antiemetic dose given and nausea and vomiting lasted <24 hrs; severe = persistent nausea and vomiting lasting ≥ 24 hrs.

days of treatment or shortly thereafter, resolving in a few days to 1 week. Audiogram changes usually involved high frequencies but occasionally did affect routine conversational hearing.

Hematologic toxicity of CIVI DDP was evaluated in 24 patients for whom blood counts were available weekly for 40 courses of treatment. The median lowest wbc count was 4.1 × $10^3/\mu l$ and the lowest median granulocyte count was 2.2 × $10^3/\mu l$ which occurred on Day 21. The median time to the next course of treatment was 31 days. Two patients developed pneumonia while neutropenic and both infectious episodes resolved with antibiotic therapy.

DISCUSSION

Although the number of patients in our series is small, these treatment results suggest activity of CIVI DDP in disseminated basal cell carcinoma. Squamous cancer of the head and neck and cervix has been shown to be sensitive to DDP by other dose schedules (2,3) so it is not unexpected that responses occurred with CIVI DDP. In 11 patients with breast cancer no responses were seen, compatible with the lack of response demonstrated when DDP is given by rapid infusion (9).

With the notable exception of nausea and vomiting, the toxicity of CIVI DDP was generally similar to that seen with other DDP schedules given at the same dose. Nephrotoxicity has been seen in 5%–15% of patients given DDP by other schedules and was not diminished by CIVI in our study (1,4). Similarly, the audiotoxicity of DDP was not altered to any major extent by CIVI. Hematologic toxicity of the CIVI regimen was generally mild and well-tolerated. The primary difference in toxicity seen with CIVI DDP was the reduced severity of nausea and vomiting. Gastrointestinal upset was moderate or severe in only about 33% of our patients. This is in contrast to our experience with rapid infusion of DDP where severe nausea and vomiting is seen in a majority of patients which discourages patients from accepting further therapy.

We conclude that CIVI of high-dose DDP can be given safely without vigorous mannitol or fluid diuresis, and that the toxicity observed is generally similar to that of the rapid infusion schedule except that nausea and vomiting is significantly reduced. The reduction in gastrointestinal toxicity may be important for patients who refuse further DDP therapy, despite antitumor benefit, because of this toxicity. Because of the good responses seen in patients with basal and squamous cancer, continued exploration of DDP alone or in drug combinations for treatment of these tumors is warranted.

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Chemotherapy for Colorectal Cancer With a Combination of 5-Fluorouracil, Mitomycin C, Adriamycin, and Cytosine Arabinoside: A Pilot Study[1,2]

Brief Reports
and
Preliminary
Communications

Stephen Davis* and Young K. Park[3]

SUMMARY

Fifty-four patients with clinical evidence of residual colorectal adenocarcinoma following surgical removal of the primary tumor were treated with a combination of 5-fluorouracil, mitomycin C, adriamycin, and cytosine arabinoside. The objective response rate in these patients was 17%. Bone marrow suppression was the major toxic effect. These data fail to show an advantage of this combination therapy over previously described chemotherapeutic regimens.

[Cancer Treat Rep 62:1557–1559, 1978]

5-Fluorouracil (5-FU) and mitomycin C, used as single agents, have been shown to have an antitumor effect against gastrointestinal (GI) carcinoma (1). However, no single agent used in therapy for colorectal carcinoma has shown a response rate of > 20% (1). Regretably, even in responders, the median response duration is brief and has added nothing to survival rates regardless of the extent of disease (2). Colorectal carcinoma, therefore, still remains a major oncologic challenge.

In 1968, Hoshino et al (3) reported that the combination of mitomycin C, 5-FU, and cytosine arabinoside (MFC) had a significant synergistic effect in the treatment of L1210 ascitic-bearing mice. This synergistic effect was confirmed by Kreis (4) using the P815 solid and ascites tumors. Subsequent clinical trials, however, of the MFC regimen in patients with GI malignancies have shown widely contrasting results (5–7). In colorectal cancer, the overall response rate was reported to be from 8% to 60%. Adriamycin, which inhibits RNA and DNA synthesis (8,9), appears to be efficacious in the treatment of some GI malignancies, especially gastric carcinoma (1,10). Although minimally effective as a single agent, adriamycin has not been adequately evaluated in a multimodal protocol for colorectal carcinoma; it was therefore used in conjunction with MFC in untreated cases of colorectal carcinoma. The results of this pilot study are reported.

METHOD AND MATERIALS

Patient Selection

A total of 54 patients who had histologically confirmed adenocarcinoma of the colon and rectum were entered in the study. All patients had undergone surgical removal of the primary lesion and had not received prior chemotherapy. All patients were required to be ambulatory with at least a 40% Karnofsky performance status at the time of entry in this study. Patients had clinically detectable disease measurable either through physical examination of masses or through roentgenographic techniques. Patients without clinically measurable disease were excluded from evaluation.

Treatment

5-FU (300 mg/m^2), mitomycin C (3 mg/m^2), and cytosine arabinoside (70 mg/m^2) were given iv on Days 1, 4, and 8, and weekly thereafter. Adriamycin (30 mg/m^2) was given iv on Day 1 and every 3 weeks thereafter.

At the start of the treatment, all patients were evaluated for performance status and measurable disease was recorded serologically or by physical examination, chest roentgenogram, and isotopic scans of liver and bone. Cbc counts were performed at the time of admission to the study and weekly thereafter.

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[3] Oncology-Clinical Immunology Section, Veterans' Administration Hospital, East Orange, NJ, and the CMDNJ-New Jersey Medical School, Newark.

**Reprint requests to:* Stephen Davis, MD, Oncology-Clinical Immunology Section, Veterans' Administration Hospital, East Orange, NJ 07019.

Treatment was continued until disease progression occurred. Adriamycin was omitted from chemotherapy when the total cumulative dose reached 550 mg/m^2. Patients were evaluated for response at appropriate intervals with the relevant chemical, laboratory, radiographic, and isotopic techniques.

An objective response was recorded if all the following criteria were met: (*a*) a reduction by at least 50% of the longest perpendicular diameters of clearly measurable areas of known malignant disease; (*b*) no increase in the size of other malignant areas; and (*c*) no evidence of new malignant disease.

RESULTS

Toxic Effects

Significant side effects among the 54 patients treated were bone marrow suppression (100%), alopecia (55%), and nausea, vomiting, and diarrhea (90%). Hematologic toxicity was marked. Severe leukopenia (< 1000 cells/mm^3) or thrombocytopenia (< 20,000 cells/mm^3) was observed in six patients. One death was ascribed to chemotherapy. No changes in liver, cardiac, or kidney functions were attributed to this drug regimen.

Therapeutic Benefits

A positive objective response rate of 17%, according to our previously stated criteria, was seen in patients with grossly detectable disease. Patients whose clinical status improved without evidence of disappearance of tumor were considered treatment failures. The median response duration was 5.2 months (range, 1–12 months). The median survival was 9.6 months in responders and 8.2 months in nonresponders.

DISCUSSION

In this uncontrolled pilot study involving a four-drug combination regimen, a relatively small percentage of patients (17%) responded to treatment.

In most studies, the overall response rate of 5-FU in colorectal carcinoma averaged 21% (1), regardless of various doses, routes, or schedules (2). In previous combination chemotherapy with 5-FU and mitomycin C (11,12) or MFC (5–7) there was a wide divergence of results as shown in table 1. The discrepancy in these reported studies and ours could be the result of patient selection. Actually, in the majority of these trials, patients who had previously failed to respond to 5-FU or other antitumor agents were placed in the study; in our study no patients who received previous therapy were included. Also, the total number of evaluable patients with colorectal carcinoma in previous studies seemed to be insufficient.

TABLE 1.—Previously reported chemotherapy response rates using various combinations of mitomycin C, 5-FU, and cytosine arabinoside (MFC) for colorectal carcinoma

Drug combination	No. of responses/ No. of evaluable patients	Response rate (%)	Ref No.
5-FU + mitomycin C	3/17	18	11
5-FU + mitomycin C	9/20	45	12
MFC	9/15	60	5
MFC	3/37	8	7
MFC	5/21	24	6

In this report, we presented 54 patients with colorectal carcinoma who were previously untreated. Although this is an uncontrolled study, the response rate, median response duration, and survival for MFC plus adriamycin seem to be no better than with 5-FU alone (2). In conclusion, because of the limited efficacy and significant toxicity, we cannot, at the present time, recommend the use of MFC plus adriamycin for patients with colorectal carcinoma.

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Symptomatic Hypocalcemia Following Combined Calcitonin and Mithramycin Therapy for Hypercalcemia Due to Malignancy[1]

Brief Reports and Preliminary Communications

Jose F. Caro,* Anatole Besarab, and Joseph A. Glennon[2]

SUMMARY

Three patients with malignant disease received the usual recommended doses of mithramycin and calcitonin, either concurrently or concomitantly, because of severe life-threatening hypercalcemia. All three patients developed severe, symptomatic hypocalcemia. The mechanisms for this phenomenon are discussed. A possible synergism between calcitonin and mithramycin may prove to be hazardous in such patients and this possibility must be kept in mind when these agents are being considered as a combination treatment for hypercalcemia.

[Cancer Treat Rep 62:1561–1563, 1978]

Three patients with hypercalcemia due to malignancy were treated with recommended doses of calcitonin (CT) and mithramycin (MT); all three sustained severe, symptomatic hypocalcemia. This apparent synergism between CT and MT should be considered when it is planned to treat hypercalcemic patients with CT and MT concomitantly.

CASE REPORTS

Patient 1.—A 59-year-old woman with untreated multiple myeloma who was diagnosed 2 years previously was admitted for evaluation of somnolence, anorexia, weakness, and back pain of 2 weeks' duration. She was confused and dehydrated and had severe lumbo-sacral pain to percussion. Laboratory tests revealed the following: serum calcium, 17.1 mg/dl (normal = 8.5–10.5 mg/dl); phosphorus, 4.9 mg/dl (normal = 2.5–4.5 mg/dl); alkaline phosphatase, 91 units/ml (normal = 30–115 units/ml); serum creatinine, 2.5 mg/dl; BUN, 21 mg/dl; and albumin, 4.3 g/dl (normal = 3.3–5.2 g/dl). Skull roentgenograms demonstrated multiple osteolytic lesions; radiographs of the spine and pelvis showed generalized osteoporosis. This patient's hypercalcemia was initially treated with saline infusion, prednisone, and im CT at a dose of 6 units/kg of body weight daily. No response was noted and on the sixth hospital day, 25 μg/kg of body weight of MT was administered as a single dose and CT was discontinued. On Day 9, transient symptomatic hypocalcemia developed associated with Trousseau's and Chvostek's signs. The total calcium level was 6.2 mg/dl, the albumin level was 4.1 g/dl, and the creatinine and BUN levels were unchanged. She did not receive calcium supplementation, and by the 15th day hypercalcemia reappeared (calcium level, 15 mg/dl). The patient was re-treated with MT alone with a return of normocalcemia by 48 hours. Subsequently, melphalan was started.

Patient 2.—A 59-year-old woman with a known adenocarcinoma of the breast who had received combination chemotherapy (cyclophosphamide, methotrexate, and 5-fluorouracil) 2 months previously was admitted with symptoms of lethargy and dehydration. Laboratory tests revealed the following: total serum calcium, 16 mg/dl; phosphorus, 3.7 mg/dl; alkaline phosphatase, 400 units/ml; creatinine, 4 mg/dl; BUN, 80 mg/dl; and albumin, 3 g/dl. A bone survey and scan revealed extensive osteolytic metastases to the skeleton. Hypercalcemia did not respond to forced saline diuresis and furosemide. The patient was then treated with MT (25 μg/kg as a single dose) and CT (6 units/kg/day) for 4 days. Symptomatic hypocalcemia developed on the fifth hospital day, at which time the serum calcium had fallen to 5.8 mg/dl, creatinine to 2.1 mg/dl, BUN to 33 mg/dl, and serum albumin to 2.9 g/dl. The hypocalcemia was treated with 500 mg of elemental calcium iv daily. On the eighth hospital day, the patient developed fever to 40°C, became septic due to *Klebsiella* pneumonia, developed shock, and died on the tenth hospital day despite the administration of appropriate antibiotics.

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[2] Department of Internal Medicine, Jefferson Medical College, Thomas Jefferson University Hospital, Philadelphia, Pa.

**Reprint requests to:* Jose F. Caro, MD, Endocrine Unit, Department of Medicine, Rochester University, Rochester, NY 14620.

Patient 3.—A 52-year-old woman admitted for evaluation of lethargy, severe weakness, and bone pain of 1 week's duration was noted to have a total serum calcium level of 20 mg/dl. Ultrafiltrable calcium measured with an Amicon cone under mineral oil was 10.7 mg/dl (normal = 4.5–5.5 mg/dl). Other studies disclosed a phosphorus of 5 mg/dl, alkaline phosphatase of 146 units/ml, creatinine of 3 mg/dl, BUN of 82 mg/dl, albumin of 4.3 g/dl, and normal serum protein electrophoresis. The urine contained 1922 mg of protein/24 hours with electrophoresis revealing lambda-type protein. A skeletal roentgenographic survey disclosed diffuse osteoporosis. Sternal bone marrow aspiration and biopsy revealed replacement of the bone marrow by plasma cells (95% of marrow cells). Initial treatment of her hypercalcemia with forced saline infusion, large doses of furosemide, and administration of hydrocortisone failed; her serum calcium persisted at > 20 mg/dl for 48 hours. Iv MT (25 μg/kg) was administered on the third hospital day as a single dose. Because of her persistent, stuporous mental state and renal impairment, an attempt to reduce her serum calcium level more rapidly was deemed necessary. Im CT (4 units/kg/day) was instituted. Normocalcemia was achieved on the tenth hospital day at which time CT was discontinued. Symptomatic hypocalcemia developed on the 22nd hospital day. Laboratory tests revealed the following: ultrafiltrable calcium, 2.5 mg/dl; total calcium, 5.5 mg/dl; magnesium, 0.6 mg/dl (normal = 1.3–3 mg/dl); albumin, 3.3 g/dl; creatinine, 1.2 mg/dl; and BUN, 26 mg/dl. The parathyroid hormone (PTH) level, measured by a carboxyterminal assay, was undetectable. Five hundred milligrams of iv elemental calcium was followed by supplementation of the patient's diet with 2.5 g/day of elemental calcium. The patient became eucalcemic and was able to maintain a normal calcium concentration. Chemotherapy was then started.

DISCUSSION

Since severe hypercalcemia in our three patients failed to respond to forced saline infusion despite urine outputs of > 3 liters/day, therapeutic agents that specifically decreased bone resorption were administered. CT at the recommended doses produces a mean decrease in serum calcium of approximately 2.5 mg/dl (1). More severe degrees of hypercalcemia may not respond adequately to this agent alone which was noted in our first patient. However, hypocalcemia did develop in this patient 5 days after MT was instituted and CT was discontinued. A similar pattern was observed in the other two patients; hypocalcemia developed 4 and 16 days after the concurrent administration of CT and MT.

The severe hypocalcemia that developed in all three patients is probably due to, in large measure, drug synergism and not totally the result of other factors. First, only one patient (No. 2) had hypoalbuminemia of moderate severity. The nomogram suggested by Orell (2) of reducing calcium by 1 mg for each gram of albumin does not explain the profound hypocalcemia noted in these patients. Second, azotemia was present in varying degrees at admission in each of the patients. Initially, they were treated with saline-induced diuresis which probably corrected the azotemia to some degree. With renal insufficiency, hypocalcemia does not appear until the glomerular filtration rate falls below 25 ml/minute (3). However, enhancement of MT's hypocalcemic effect by azotemia may have contributed to the hypocalcemia seen in these patients. Third, there was no clinical or laboratory evidence of pancreatitis. Fourth, hypocalcemia as a complication of cancer is rare. It has been reported to occur spontaneously in lung cancer, after successful treatment of carcinoma of the prostate, and during treatment of breast carcinoma with extensive osteoblastic metastases (4).

In our three patients, the time course of hypocalcemia suggests the synergistic effect of drugs on bone. A possible explanation can be obtained from an analysis of the Rasmussen and Bordier model for bone formation (5). In this model, bone remodeling is a coupled process determined by the relative degrees of osteoclastic and osteoblastic activity. The net effect of CT is the inhibition of osteoclast formation which decreases bone calcium release and stimulation of new bone formation by osteoblasts which deposit calcium in bone matrix. MT probably decreases bone resorption independent of PTH activity by inhibition of DNA-dependent RNA synthesis which inactivates osteoclasts (6). MT, when used alone, has only a transient hypocalcemic effect due to secondary hypocalcemic stimulation of PTH secretion which increases osteoclast production. This may be why hypocalcemia is rare after MT therapy alone and was noted in only one of 32 patients studied by Perlia et al (7). CT administration during MT treatment may blunt or abolish the PTH-mediated rebound to hypocalcemia leading to a prolonged hypocalcemic response to MT. This might explain the development of hypocalcemia in our patients, but the duration and refractory nature of the hypocalcemia which was encountered are surprising. Restoration of serum calcium to normal may require the recruitment of mesenchymal cells into the osteoclast pool since the effect of MT and CT is to markedly favor bone formation over bone resorption. This recruitment process requires time (5). In addition, azotemia may have enhanced the effect of MT and CT.

Alternatively, hypomagnesemia may have been responsible for the prolonged hypocalcemic response due to MT osteoclast inactivation. Short-term CT administration increases magnesium excretion (8) which may aggravate pre-existing hypomagnesemia. Magnesium deficiency, so induced, may result in defective cyclic adenosine monophosphate generation in the parathyroid gland and PTH target organs leading to both impaired PTH secretion and PTH end organ insensitivity (9). Severe hypocalcemia and hypomagnesemia in one of our patients coincided with undetectable PTH levels suggesting that hypomagnesemia did interfere with PTH secretion possibly contributing to the hypocalcemia encountered. Serum magnesium levels were not measured in our other two patients. The interval between treatment and onset of hypocalcemia, however, was much shorter (4 and 5 days, respectively), and it is unlikely that significant magnesium depletion could have occurred in such a short time. However, hypomagnesemia could have been present prior to any treatment contributing to hypocalcemia that developed.

These three case reports emphasize the possible hazard of concomitant treatment with CT and MT. The order of their administration does not appear to influence the development of symptomatic hypocalcemia which may be severe and prolonged. If these agents together are to be considered for the treatment of hypercalcemia, modified dosage schedules may have to be developed, the dose must be changed or discontinued if calcium levels fall rapidly, and the serum magnesium levels must be monitored carefully.

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Amphotericin B and Combination Chemotherapy in the Treatment of Refractory Metastatic Breast Carcinoma and Sarcoma [1,2]

Allan N. Krutchik, Aman U. Buzdar,* George R. Blumenschein, and Joseph G. Sinkovics [3,4]

SUMMARY

Nineteen patients resistant to an adriamycin-containing regimen were treated with 4 days of amphotericin B and the same adriamycin-containing chemotherapy regimen that had been given without amphotericin B. Of the 14 evaluable patients (11 with breast carcinoma and three with sarcoma), one patient with breast carcinoma achieved a partial remission and a second patient with breast carcinoma remained stable. All other patients developed progressive disease. Both hematologic and nonhematologic toxicity were significantly increased when amphotericin B was added to chemotherapy.

[Cancer Treat Rep 62:1565–1567, 1978]

A common problem in the treatment of neoplastic disease is the development of drug resistance in a particular tumor. Amphotericin B has been shown to overcome this drug resistance in selected tumors. The postulated mechanism of action of sensitizing resistant tumor cells is by enhancement of drug uptake by tumor cells by pretreatment with amphotericin B (1). We previously reported the results of a pilot study of six patients (three with breast carcinoma and three with sarcoma) (2). This is an updated report with additional patients showing one objective response (7%) in 14 patients.

PATIENTS AND METHODS

Between April and December 1977, 19 consecutive patients (14 with breast carcinoma and five with sarcoma) were treated with a combination of amphotericin B plus an adriamycin-containing regimen. Patients were eligible for the study if they had measurable metastatic disease and had shown progression of tumor growth after at least two courses of an adriamycin-containing regimen. The patients were to receive the same doses of chemotherapy as they had received without pretreatment with amphotericin B. The patient characteristics are shown in table 1.

The treatment consisted of amphotericin B given at doses of 7.5 mg/m² iv on Day 1, 15 mg/m² iv on Day 2, and 30 mg/m² iv on Days 3 and 4. The rationale for this schedule has been reported elsewhere (3). Chemotherapy regimens are shown in table 1 and were given on Day 4 immediately after the infusion of amphotericin B was completed. In those patients receiving DTIC, the drug was given for an additional 4 days.

Amphotericin B was given iv in 5% dextrose in water during a 6-hour period. Patients were given Benadryl and acetaminophen to control rigors and fever. All evaluable patients received an average of two courses (range, one to five courses) of chemotherapy following the addition of amphotericin B. Chemotherapy and amphotericin B were given every 3 weeks. A dose reduction of 20% of the adriamycin-containing regimen was made in patients if the granulocyte count dropped to $< 1000/\text{mm}^3$ or the platelet count to $< 75{,}000/\text{mm}^3$ or if an infection developed during the course of treatment.

The following criteria were used to evaluate response: (*a*) *complete response*—complete disappearance of all objective evidence of measurable disease with no new disease, (*b*) *partial response* (*PR*)—a decrease of ≥ 50% in the products of two diameters of measured lesions without the appearance of new lesions, (*c*) *stable disease*—a decrease of < 50% in

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[3] Department of Medicine, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

[4] We thank Dr. Stuart W. Leafstedt of Sioux City, Iowa, for his assistance in the care and management of patients in the study.

**Reprint requests to:* Aman U. Buzdar, MD, Department of Medicine, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, Tex 77030.

TABLE 1.—Patient characteristics

Total No. of patients	19
Age in yrs	
Median	49
Range	20–65
No. of evaluable patients	14
Breast carcinoma	11
Sarcoma	3
Osteosarcoma	1
Epithelioid sarcoma	1
Malignant fibrous histiocytoma	1
Menopausal status (breast carcinoma patients)	
Premenopausal	0
Postmenopausal	11
Dominant lesion	
Soft tissue	1
Visceral	8
Bone	5
No. of metastatic sites	13
1 or 2	13
≥ 3	1
Performance status (Karnofsky)	
Median	80
Range	50–90
Previous therapy before amphotericin B was added	
Breast carcinoma patients	
5-Fluorouracil-adriamycin-cyclophosphamide	8
Adriamycin-cyclophosphamide	1
Adriamycin-vincristine	1
Adriamycin	1
Sarcoma patients	
Adriamycin-DTIC	2
Adriamycin	1

the product of diameters of measured lesions without the appearance of new lesions for a period of 8 weeks, and (*d*) *progression*—an increase of $\geq 25\%$ in the products of measured lesions with or without the appearance of new lesions.

RESULTS

Fourteen of 19 treatment trials (11 patients with breast carcinoma and three patients with sarcoma) were considered evaluable. Three patients died of progressive disease within 1 week of treatment and were inevaluable. One patient was inevaluable because of nonmeasurable disease and it was considered too early to evaluate the remaining patient. One patient with breast carcinoma achieved a PR for a duration of 4 months. This patient was a 49-year-old woman who was perimenopausal with an estrogen-receptor negative tumor. Before the addition of amphotericin B, the patient's total dose of adriamycin was 220 mg/m^2. The sites of disease were the lung and soft tissue (chest wall and cervical adenopathy). A second patient with breast carcinoma achieved stable disease for 8 weeks. All other patients failed to show an objective response.

Toxicity

The median lowest absolute granulocyte and platelet counts and hemoglobin levels are shown in table 2. These were compared to the median lowest counts that occurred in the last course of the adriamycin-containing chemotherapy regimen given without amphotericin B. There was a significant depression of the granulocyte count and hemoglobin level when amphotericin B was added to adriamycin in the first course ($P < 0.01$). There was no significant effect on the platelet counts in the first course when amphotericin B was added to the adriamycin regimen ($P > 0.05$). Patients with hemoglobin levels < 8 g/100 ml received transfusions of packed rbcs. None of the patients required platelet transfusion. Three patients developed infections (one patient with septicemia and two with urinary tract infections). There were no toxic deaths as a result of infection. Transient azotemia was observed in four patients. None of the patients developed acute renal failure. Additional toxic effects are noted in table 3. One patient died from congestive heart failure. She received a total dose of 480 mg/m^2 of adriamycin and had received left chest wall and peripheral lymphatic irradiation. A second patient developed signs of congestive heart failure when her total dose of adriamycin was 456 mg/m^2. This patient did not receive any precardial radiation therapy. Both patients had received cyclophosphamide concomitantly with adriamycin.

DISCUSSION

Previous studies have demonstrated that amphotericin B with various nitrosoureas resulted in antitumor responses in tumors refractory to conventional chemotherapy (3,4). The responsive tumors consisted of breast carcinoma, lung carcinoma, and thyroid carcinoma. Presant et al have shown that patients who have failed to respond to a regimen of adriamycin, BCNU, and cyclophosphamide did respond to this regimen when it was administered sequentially after amphotericin B. The tumors responding in that study were breast carcinoma, acute myelomonocytic leukemia, and multiple myeloma (1).

In this study we have demonstrated one PR in a patient with breast carcinoma who had both visceral (lung) and soft tissue (cervical adenopathy and chest wall) disease sites. Additionally, one patient

TABLE 2.—Hematologic toxicity

	Last course of adriamycin regimen before amphotericin B	Course 1 with amphotericin B	Course 2 with amphotericin B
Median lowest—			
Granulocyte count (cells/mm^3)	2150	900	800
Range	955–9800	400–3200	357–2400
Platelet count (cells × 10^3/mm^3)	181	72	65
Range	63–600	19–398	24–377
Hemoglobin level (g/100 ml)	10.8	7.8	6.5
Range	7.9–14.2	7.0–13.2	5.8–7.7

TABLE 3.—Nonhematologic toxicity

Toxic effect	No. of patients
Congestive heart failure	2
Temperature elevation	9
Rigors	5
Hypotension	1
Infection	3
Bronchospasm	1

with breast carcinoma maintained stable disease for a period of 8 weeks. Of interest was that in the only patient showing a response, tumor resistance was demonstrated initially prior to the addition of amphotericin B; in all other patients, an antitumor response was present initially with tumor resistance developing subsequently before amphotericin B was employed. There were no responses among the sarcoma patients. The only other reported sarcoma patient treated with amphotericin B and chemotherapy was a patient with rhabdomyosarcoma who developed progressive disease while receiving the regimen (3). Unlike other reports (1), there was a significant increase in the myelosuppressive toxicity of drugs with the addition of amphotericin B. Two patients in this group developed congestive heart failure although other risk factors (irradiation and cyclophosphamide) might have been responsible for this effect; the possibility of amphotericin B potentiating the myocardial damage cannot, however, be ruled out.

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Brief Reports and Preliminary Communications

Phase I Study of Pyrazofurin in Refractory Acute Myelogenous Leukemia [1,2]

William R. Vogler* and Phyllis D. Trulock[3] ***(Writing Committee for the Southeastern Cancer Study Group)***

SUMMARY

Pyrazofurin was administered to 17 patients with refractory acute myelogenous leukemia in 5-day courses every 2–3 weeks. Doses ranged from 30 to 60 mg/m^2/day. Severe stomatitis and dermatitis occurred at doses effective in reducing the leukocyte count (45 mg/m^2). Reduction of the dose to 30 mg/m^2 resulted in less toxicity and less chemotherapeutic effect. These results indicate that at tolerable doses given as described, pyrazofurin had little antileukemic activity in acute myelogenous leukemia.

[Cancer Treat Rep 62:1569–1571, 1978]

Pyrazofurin is a new antipyrimidine drug produced in fermentation broths by *Streptomyces candidus* which has antitumor activity in animals (1). Clinical trials in patients have been reported (2–4). The drug has been given in consecutive daily doses, in weekly injections, and in 5-day courses. The dose causing toxicity varied, depending on the method of administration. In general, toxicity consisted of neutropenia, thrombocytopenia, stomatitis, and dermatitis. Since this agent had limited trials in acute leukemia, the Southeastern Cancer Study Group began a phase I trial in refractory acute leukemia.

Criteria for Patient Selection

The criteria for patient selection required a diagnosis of acute leukemia and failure of previous therapy. Patients giving informed consent had physical examinations, blood cell counts, hepatic function tests (alkaline phosphatase, SGOT, serum protein, and bilirubin), and renal function tests (BUN and creatinine clearance) prior to treatment.

Dosages and Schedules

The treatment plan consisted of escalating doses by 15 mg/m^2 in groups of three patients beginning at 45 mg/m^2/day × 5 days. Courses were repeated every 2 weeks. Once a therapeutic or maximum toxic dose had been achieved, 12 patients were to be treated at that dose level. Patients receiving at least two courses of treatment were considered to have had an adequate therapeutic trial. Patients receiving one course of treatment were evaluable for toxicity. Blood cell counts were followed at least twice weekly, usually daily, and blood chemistries were determined weekly.

Toxicity Evaluation

Grading of hematopoietic toxicity was as follows: *grade 1* (a fall in hemoglobin concentration of 3–4.9 g/dl, a fall in granulocyte count from > 3000 to $< 1500/\mu l$ or from > 750 to $< 200/\mu l$, and a fall in platelet count from $> 130{,}000$ to $< 100{,}000/\mu l$, from $> 100{,}000$ to $< 50{,}000/\mu l$, or from $> 50{,}000$ to $< 10{,}000/\mu l$); *grade 2* (a fall in hemoglobin concentration of > 5 g/dl, a fall in granulocyte count from > 3000 to $< 750/\mu l$ or from > 1500 to $< 200/\mu l$, and a fall in

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[3] The following members of the Southeastern Cancer Study Group participated in this study: *Richard A. Gams, George A. Omura,* and *Marcel Conrad* (University of Alabama School of Medicine); *Issac Esseesse* (Veterans' Administration Hospital, Brooklyn); *Harold Silberman* (Duke University School of Medicine); *William R. Vogler* and *Elliott F. Winton* (Emory University School of Medicine); *Bruce Stechmillar* (University of Florida School of Medicine); *Philip DeSimone* (University of Kentucky School of Medicine); *William H. Knospe* (Rush-Presbyterian-St. Luke's Medical Center); and *Gononwy O. Broun* and *Paul J. Petruska* (St. Louis University School of Medicine).

**Reprint requests to:* William R. Vogler, MD, Emory University School of Medicine, 718 Woodruff Memorial Bldg, Atlanta, Ga 30322.

TABLE 1.—Toxicity and antileukemic effects

First course dose (mg/m^2 × 5 days)		No. of patients	Mucositis		Dermatitis	Decrease in circulating blast cells
			Grade 1	Grade 2		
		Following 1st course of treatment				
30		8	3	0	1	4
45		9	0	5	4	6
		Following 2nd course of treatment				
Prior dose	Current dose					
30	30	5	1	1	0	1
45	30	2	1	0	0	1
30	45	1	0	0	0	0
45	45	2	0	2	1	1
45	60	1	0	1	1	1
		Following 3rd course of treatment				
Prior doses	Current dose					
30–30	45	1	1	0	1	1
45–45	45	1	1	0	0	1

platelet count from $> 130,000$ to $< 50,000/\mu l$ or from $> 100,000$ to $< 10,000/\mu l$); *grade 3* (a grade 2 toxicity associated with a life-threatening complication).

Mucositis was graded as follows: *grade 1* (mild mucositis lasting 1–3 days); *grade 2* (moderate mucositis with ulcerations lasting 3–6 days); and *grade 3* (severe mucositis with extensive ulcerations lasting > 5 days).

Gastrointestinal toxicity was graded as mild, moderate, or severe depending upon the degree of the nausea and vomiting and the occurrence of diarrhea. Grade 1 hepatic toxicity consisted of a rise in SGOT or alkaline phosphatase of 50%, and grade 2 consisted of a rise of 100% not attributable to disease. A rise to above normal in creatinine or BUN or a fall in creatinine clearance to < 60 ml/minute was considered evidence of renal toxicity.

Responses in acute leukemia were judged by criteria previously reported (5).

RESULTS

The results of the treatment are summarized in table 1. Eighteen patients were entered in the study. Except for one patient who was in the blastic phase of chronic granulocytic leukemia, all had acute myeloblastic leukemia. All patients had received previous therapy and, with the exception of the patient with blastic phase chronic granulocytic leukemia, had had previous remissions. Therapy had consisted of cytosine arabinoside in combination with daunorubicin and/or 6-thioguanine. In addition, 12 patients had been treated with a combination of 5-azacytidine and β-2′-deoxythioguanosine. The ages ranged from 22 to 67 years with an average age of 46 years. One patient was judged inevaluable because hydroxyurea was added on the fourth day of pyrazofurin treatment.

Nine patients began receiving treatment at an initial dose of 45 mg/m^2/day. One patient died of sepsis on Day 3. Six patients demonstrated a transient reduction, two showed no change, and one had a rise in circulating blast cells following the first course of treatment.

Although one patient had a dramatic fall in circulating blast cells from 390,000 to 8200/μl within 5 days, she died of infection that day. The reduction in circulating blast cells in the other five patients varied from 15% to 65% of the pretreatment values. The nadir was usually on Days 8–10, but varied from Day 7 to 15.

Five patients received a second course of pyrazofurin beginning on Day 14. In two patients the dose was unchanged, in two it was reduced to 30 mg/m^2, and in one it was increased to 60 mg/m^2. One patient received three courses at a dose of 45 mg/m^2. Two courses were given without toxicity and a transient fall in blast cells occurred after each course. Grade 1 mucositis and mild nausea and vomiting occurred after the third course. Another patient received two courses at a dose of 45 mg/m^2 and developed grade 2 mucositis and hepatic toxicity after each course and dermatitis after the first course. Only a transient fall in blast cells was observed. Reducing subsequent doses to 30 mg/m^2 resulted in less mucositis and dermatitis in two patients as well as a lesser effect on circulating blast cells. One patient with no toxicity at a dose of 45 mg/m^2 developed grade 2 mucositis and dermatitis when given a dose of 60 mg/m^2. The transient fall in circulating blast cells was similar in both instances.

Due to the high frequency of mucositis and dermatitis observed at 45 mg/m^2/day, this dose was reduced. Eight patients were started at a dose of 30 mg/m^2/day which resulted in less toxicity. Only one patient developed grade 1 mucositis and dermatitis. Two patients showed a transient fall in circulating blast cells. One was a patient in the blastic phase of chronic granulocytic leukemia whose leukocyte count dropped from 30,000 to 3800/μl by Day 10. The count rose following a second course. The other patient showed only a 55% reduction in blast cells. Five patients received a subsequent course of 30 mg/m^2 beginning on Day 14; two of these patients developed mucositis (grades 1 and 2) and one developed dermatitis. One patient received three courses of pyrazofurin. The first two courses were given at 30-mg/m^2 doses and the third course was given at a 45-mg/m^2 dose. There was a fall in the leukocyte count after each course of treatment. Following the second course the bone marrow was less cellular with some increase in granulocytic differentiation. However, the patient requested that treatment be delayed until Day 25 and the marrow again was hypercellular with 81% blast cells. Dermatitis and mucositis followed each course of treatment.

Because of the leukemic status there were only three patients in whom hematologic toxicity could be evaluated. One patient each had a grade 1 and a grade 2 granulocyte toxicity and one had a grade 2 platelet toxicity. One patient had elevation of SGOT and alkaline phosphatase after each of two courses of treatment. No renal toxicity was observed.

Thus, of 17 patients evaluable in the study, eight developed mucositis, five developed dermatitis, and ten had a transient reduction in blast cells. Eleven patients received a second course of treatment; six developed mucositis, two had dermatitis, four had a transient reduction in circulating blast cells, and one had a transient marrow improvement. Lower doses produced less severe and less frequent toxicity. Toxicity usually lasted as long as the transient reduction in circulating blast cells. The mucositis and dermatitis usually appeared on about the fourth day of treatment and lasted 3–6 days. The rash was usually generalized, maculopapular, and frequently nonpruritic. Healing was usually associated with desquamation.

DISCUSSION

Biochemical studies have demonstrated that pyrazofurin is a potent inhibitor of de novo pyrimidine biosynthesis competitively inhibiting orotidylate decarboxylase (6). Phosphorylation to monophosphate and polyphosphate derivatives has been demonstrated in human leukemic cells and rbcs. Following iv injection of a bolus dose of pyrazofurin, the urinary excretion of orotidine remained elevated for 9–12 days (7). The recovery of orotic acid metabolism in leukemic cells took several days but did not result in a significant decrease in circulating blast cells. When given as a continuous infusion for 6 days at a dose of 250 mg/m^2/day, significant reduction of leukemic cells in the blood and marrow occurred, but with severe toxicity. Infusions reduced to 72 hours were better tolerated, but the leukemia progressed in four patients treated this way (2). Since very little hematologic toxicity was observed with weekly bolus doses (2), we chose a 5-day course of treatment with the hope of obtaining greater marrow toxicity in leukemic patients. Unfortunately, other toxicities proved to be the limiting factor.

In conclusion, the maximum tolerated dose of pyrazofurin given by iv bolus daily × 5 days is 30 mg/m^2. This dose is ineffective in reducing blast cell counts. Mucositis and dermatitis are the major side effects. As a single agent pyrazofurin is relatively inactive in refractory acute myelogenous leukemia.

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Brief Reports and Preliminary Communications

Phase II Evaluation of Pyrazofurin in Patients With Metastatic Sarcoma[1,2]

Richard J. Gralla,* Peter P. Sordillo, and Gordon B. Magill[3,4]

Pyrazofurin is a C-ribonucleoside antibiotic with demonstrated activity against experimental tumor models, including Walker 256 carcinosarcoma (1,2). After activation to the monophosphate, the drug inhibits orotidylic decarboxylase (1,3). A weekly administration schedule was selected since pharmacokinetic studies revealed that a single iv dose of pyrazofurin caused a $> 95\%$ inhibition of ^{14}C-labeled orotic acid metabolism at 24 hours and that a $> 30\%$ inhibition persisted at 9 days (3). Manageable toxicity has been noted at doses of 5–7 mg/kg (200–300 mg/m^2) (4,5) with occasional objective responses reported in patients with mycosis fungoides, acute myelocytic leukemia (3), and breast cancer (4). In published phase II studies, pyrazofurin has not shown major therapeutic activity in patients with advanced melanoma (6), carcinoma of the lung (7), or carcinoma of the large bowel (8).

MATERIALS AND METHODS

Twenty-seven patients with histologically confirmed metastatic sarcoma and objectively measurable tumor were enlisted in this trial from November 1976 to December 1977. Of the 20 adequately treated patients, the median age was 46 years (range, 29–72 years) and the median performance status on the Karnofsky scale was 60 (range, 40–90). All patients had received prior chemotherapy, and eight had received radiation therapy as well. The histologic types of diseases in these patients were as follows: seven with leiomyosarcoma, three with liposarcoma, two with synovial sarcoma, two with osteogenic sarcoma, and one each with fibrosarcoma, embryonal rhabdomyosarcoma, spindle cell sarcoma, peritoneal mesothelioma, ovarian stromal sarcoma, and hemangiopericytoma. With the exception of one patient who had only multiple subcutaneous masses, all patients had pulmonary metastases. In addition, five of these patients had measurable metastases in other sites, including subcutaneous tissue (two), abdominal lymph nodes (two), and brain (one).

An adequate trial required at least three weekly doses of pyrazofurin accompanied by dose-limiting toxicity related to mucositis or blood cell count depression. Therapeutic responses were defined as follows: *partial remission*—$\geq 50\%$ decrease of all measurable tumor masses for ≥ 1 month, and *minor response*—objective tumor decrease ($> 25\%$), but less than that required for a partial remission.

Pyrazofurin (supplied by Eli Lilly and Co, Indianapolis, Ind) was administered as a rapid iv injection beginning at a weekly dose of 5 mg/kg (200 mg/m^2) with dose increments of 0.5 mg/kg/week (20 mg/m^2) until definite but manageable toxicity occurred. The dose was attenuated to 4 mg/kg (160 mg/m^2) if the wbc count was 3000–3900/mm^3 or if the platelet count was 75,000–99,000/mm^3. The drug was withheld for 1 week if the wbc or platelet counts were less than these levels, if the hemoglobin level had fallen by ≥ 2 g/100 ml, or if the patient was unable to eat because of mucositis.

RESULTS

Table 1 outlines the toxic reactions of pyrazofurin therapy observed during this trial. As reported in prior studies (4,7), the most frequently noted toxic effect in our patients was a normochromic normocytic anemia. Of the 15 patients who had a hemoglobin decrease of ≥ 2.0 g/100 ml (median, 3.3 g/100 ml), the anemia was generally noted within 1–2 weeks after therapy was started. Dose-limiting mucositis occurred frequently, but resolved after one weekly dose of pyrazofurin was withheld. One patient had a transient generalized macular erythematous skin eruption which did not recur with further treatment. There were no episodes of drug-related septicemia or hemorrhage and no deaths occurred.

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[3] Clinical Chemotherapy Service (*R. J. Gralla*) and Solid Tumor Service (*P. P. Sordillo* and *G. B. Magill*), Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY.

[4] We wish to thank Kathleen Trainor for preparation of the manuscript and Laura Lapp for assistance with analysis of the data.

**Reprint requests to:* Dr. Richard J. Gralla, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

TABLE 1.—Toxic reactions of 20 patients treated with pyrazofurin

Toxic effect	No. of patients
Mucositis	14
Nausea and vomiting	9
Rash	1
Platelet count nadir (cells/mm^3)	
≥ 150,000	10
100,000–149,999	2
50,000–99,999	6
25,000–49,999	2
Wbc count nadir (cells/mm^3)	
≥ 4000	15
3000–3999	2
2000–2999	3
1000–1999	0
Hemoglobin decrease (g/100 ml)	
0–1.9	5
2.0–2.9	4
3.0–3.9	6
≥ 4.0	5

The 20 adequately treated patients received a median of five doses of pyrazofurin (range, three to 14 doses). No major antitumor activity occurred. However, one patient with a liposarcoma (Karnofsky performance status = 80) experienced a minor response which consisted of a > 50% decrease in pulmonary metastases but a < 50% shrinkage of subcutaneous masses.

DISCUSSION

Twenty-seven patients with metastatic sarcoma were treated with weekly doses of iv pyrazofurin. No patient experienced a major therapeutic response; dose-limiting toxic effects included mucositis and anemia not associated with hemorrhage. Although routine studies (haptoglobin, reticulocyte counts, lactic dehydrogenase, bilirubin, and Coombs' tests) did not reveal evidence of hemolysis, this possibility cannot be ruled out at this time. Preliminary studies by Dyke et al[5] have indicated a decrease in survival of ^{51}Cr-labeled rbcs in a pyrazofurin-treated patient when compared with a pretreatment study.

Although all patients treated in the present trial had received prior chemotherapy, their general physical condition was good enough to allow outpatient management. With failure to achieve a partial remission in this number of patients, it can be concluded that pyrazofurin is likely to have a true major response rate of < 15% (*P* = 0.05) (9) and it would appear to have little therapeutic value in this schedule as a single agent in previously treated patients with advanced metastatic sarcoma.

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Brief Reports and Preliminary Communications

Regression of 180J Sarcoma After Treatment With Substituted Indoloquinolinones [1,2]

George S. Tarnowski,[3,*] C. Chester Stock,[3] and Emmanuel Grunberg [4,5]

Nonspecific animal tumors, such as 180 sarcoma (S180), respond readily with a complete regression to therapy with immunomodulating agents. Plant polysaccharides, endotoxins, and other high molecular weight biologic products produce regression of such tumors even when therapy starts a few days after tumor implantation (1,2). Among the low molecular weight synthetic immunomodulating chemicals, tilorone and its congeners (3), levamisole (4), and chlorphenesin (5) inhibited growth of some animal tumors, and chlorphenesin induced regression of S180.

Grunberg et al observed that therapy with 10-chloro-5-(2-dimethylaminoethyl)-7*H*-indolo[2,3-*c*]quinolin-6(5*H*)-one hydrochloride (I) inhibited growth of mouse and rat tumors (6). This initial observation was confirmed and extended in the present study.

MATERIALS AND METHODS

The S180J tumor line of S180 (2) was maintained in ascites form in CD-1 female mice from the Charles River Mouse Farms (Wilmington, Mass); for testing, 7.5×10^5 tumor cells were implanted intradermally on Day 0 into groups of six CD-1 mice. B16 melanoma and Lewis lung tumor were transplanted as trocar pieces into male C57BL/6J mice and Meth A fibrosarcoma (2) was transplanted into female (BALB/cJ × C57BL/6J)F_1 mice (Jackson Laboratory, Bar Harbor, Me).

Indoloquinolinones (supplied by Hoffmann-La Roche, Nutley, NJ) were suspended (lower doses were dissolved) in 0.5% solution of carboxymethylcellulose in saline and administered by different routes of administration to the tumor-bearing mice. The average tumor diameters and body weights of the hosts were measured every week and mice were observed for tumor regression for 42 days. Usually tumor regression took place during the second to the fifth week of tumor growth and no additional tumor regression was observed afterwards.

For the in vitro-in vivo study, suspensions of 1×10^6 S180J cells/ml were prepared in Earle's balanced salt solution, saturated at room temperature with indoloquinolinone (I), containing 200 μg/ml of the chemical. The pH of such a solution was that of saline. After incubation for 1 hour at 37°C, the percent of trypan blue-staining cells was determined, and aliquots containing 3×10^5 tumor cells were injected intradermally into groups of six CD-1 mice. The number of tumor-free mice at the end of 28 days was recorded.

RESULTS

In the first series of tests, ten doses of indoloquinolinone (I) were administered to mice bearing S180J tumors ip, one dose each day, starting on Day 4 prior to tumor inoculation in order to pretreat the tumor recipients and to treat them after the tumor implantation (table 1). With this regimen, no inhibition of tumor growth or prolongation of survival time of mice bearing tumors was observed. Toxic effects were seen only after the highest dose of 64 mg/kg/day. A dose-dependent increase in tumor regression occurred in treated mice. Three congeners of indoloquinolinone (I), 10-chloro-5-(2-aminoethyl)-(II), 10-ethyl-5-(2-dimethylaminoethyl)-(III)-7*H*-indolo[2,3-*c*]-quinolin-6(5*H*)-one, and 10-chloro-2,13-dihydro-3*H*-imidazo[1,2-*a*]-indolo-[2,3-*c*]quinoline (IV), did not inhibit tumor growth but caused a regression of tumors in mice treated with the highest tolerated doses of these chemicals.

In subsequent tests, pretreatment was omitted and therapy was started on Day 1 which consisted of ten daily doses of 16 mg/kg/day of indoloquinolinone (I); regression of S180J in 80%–90% of treated mice was observed after ip, sc, or oral administration of the chemical; tumors regressed in 58% of

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[3] Donald S. Walker Laboratory, Sloan-Kettering Institute for Cancer Research, Rye, NY.

[4] Research Division, Hoffmann-La Roche, Inc, Nutley, NJ.

[5] We wish to thank Susan Urbano, Stephen Banks, and Harry Satterwhite for their valuable assistance in the execution of this study.

[*] *Reprint requests to:* Dr. George S. Tarnowski, Donald S. Walker Laboratory, Sloan-Kettering Institute for Cancer Research, 145 Boston Post Rd, Rye, NY 10580.

TABLE 1.—Incidence of regression of S180J treated daily with indoloquinolinone (I)*

Route of administration	Therapy started on Day—	Dose (mg/kg/day)	Tumor-free surviving mice on Day 42 in test No.—†				Death rate in all tests
			1	2	3	Total	
ip	– 4	0‡	0/4	0/6	0/6	0/16	2/18
ip	– 4	0.25	0/6	1/6	—	1/12	0/12
ip	– 4	1	1/5	3/6	1/6	5/17	1/18
ip	– 4	4	2/6	4/6	3/6	9/18	0/18
ip	– 4	16	4/6	1/6	3/6	8/18	0/18
ip	– 4	64	1/1	3/5	—	4/6	6/12
ip	1	0§	1/6	1/6	—	1/12	0/12
ip	1	16	5/6	5/6	—	10/12	0/12
ip	3	16	6/6	6/6	—	12/12	0/12
ip	7	16	3/6	4/6	—	7/12	0/12
sc	1	0§	0/6	1/6	—	1/12	0/12
	1	16	4/6	5/6	—	9/12	0/12
oral	1	0§	1/6	1/6	1/6	3/18	0/18
	1	16	4/5	4/6	6/6	14/17	1/18

*On Day 0, 7.5 × 10^5 S180J cells were implanted intradermally into groups of 6 female CD-1 mice in each test; diluent: 0.5% carboxymethylcellulose in saline, 0.5 ml/dose.

†Tumor-free survivors had no palpable tumors.

‡4 doses, 1/day, on Days – 4, – 3, – 2, and – 1 and 6 doses on Days 1–6.

§6 daily doses on Days 1–6.

mice even when therapy was started on Day 7 after tumor inoculation (table 1).

Syngeneic mouse-tumor systems of B16 melanoma, Meth A fibrosarcoma, or Lewis lung tumor were not inhibited by indoloquinolinone (I), and no tumors regressed.

Cells of S180J or Meth A exposed in vitro to saturated solutions of indoloquinolinone (I) and then inoculated intradermally into mice produced the same number of tumors at the site of inoculation as control tumor cells incubated in saline. No increase in the percent of trypan blue-staining tumor cells was observed.

DISCUSSION

Regression of S180J treated with indoloquinolinones occurs 2–3 weeks after the end of therapy and is not preceded by retardation of tumor growth at the time of therapy. No direct toxicity to tumor cells in solutions of indoloquinolinone (I) was observed in short-term incubation tests. These findings suggest the modulation of immune reaction of the host to this tumor as the important factor responsible for its rejection. Not enough is known about the nature of cellular or humoral mechanisms involved in rejection of nonspecific tumors such as S180. Maeda et al (1) have presented evidence that regression of S180J treated with the polysaccharide lentinan might be due to stimulation of the T cell-dependent humoral response.

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Phase II Evaluation of Maytansine in Patients With Metastatic Lung Cancer [1,2]

Brief Reports and Preliminary Communications

Robert T. Eagan,* Edward T. Creagan, James N. Ingle, Stephen Frytak, and Joseph Rubin [3]

Maytansine (MAYT) is a naturally occurring ansa macrolide originally isolated from the East African shrub *Maytenus ovatus* (1). MAYT belongs to a new class of compounds characterized by the presence of a large macrocyclic lactone ring, frequently *N*-heterocyclic, and incorporating within it an *m*- or *p*-bridged aromatic moiety.

MAYT inhibits mitosis (metaphase), an action similar to vincristine and vinblastine (2). The drug inhibits DNA synthesis more than it does RNA or protein synthesis. It is active against a variety of in vivo tumor systems including P388 leukemia, B16 melanoma, and Lewis lung carcinoma, as well as against KB, L1210, and P388 tumors in vitro (1).

We recently reported on the results of our phase I clinical trial of an intermittent schedule for MAYT (3). In that study, we found the combination of severe nausea and vomiting, diarrhea, and liver function abnormalities to be dose-limiting toxic effects of the drug. An every-other-day schedule for three doses (ie, treatment on Days 1, 3, and 5) repeated every 4 weeks was evaluated. We concluded that 0.9 mg/m^2 iv for three doses (total, 2.7 mg/m^2) was the maximum-tolerated dose and recommended a schedule of 0.75 mg/m^2 iv for three doses (total, 2.25 mg/m^2) repeated every 4 weeks for phase II trials. After a discussion at the National Cancer Institute with other investigators who have done phase I studies of MAYT, a schedule of 0.6 mg/m^2 iv for 3 consecutive days (treatment on Days 1, 2, and 3), repeated first at 3 weeks followed by retreatment every 4 weeks, was chosen for our phase II study in patients with metastatic lung cancer. This schedule was evaluated by Cabanillas et al (4).

MATERIALS AND METHODS

Patients were considered eligible if they had histologic or cytologic evidence of metastatic squamous cell carcinoma (SQC), large cell carcinoma (LGC), small cell carcinoma (SMC), or adenocarcinoma (ACA) of the lung and had failed treatment with at least one known effective regimen unless laboratory or clinical factors prevented the use of these regimens first. An area of measurable or evaluable tumor was required.

Forty-seven patients were entered in the study and all were evaluable, at least for survival. Eleven patients had received no prior chemotherapy. Thirty-six percent of patients had Eastern Cooperative Oncology Group (ECOG) performance scores (PS) of 0–1 and 64% had PS of 2–3. Patients with a PS of 4 were not eligible for study. The pretreatment patient characteristics are displayed in table 1.

MAYT was administered by rapid iv infusion over 3–10 minutes into the side arm of a freely flowing infusion setup. The starting dose was 0.6 mg/m^2 given on 3 consecutive days (total, 1.8 mg/m^2). The first re-treatment was scheduled for 3 weeks with subsequent re-treatment scheduled for every 4 weeks. Patients were removed from the study at the first sign of disease progression.

Patients with clearly measurable indicator lesions were evaluated using standard criteria for complete regression, partial regression (PR), stability, and progression. Patients with evaluable, but not clearly measurable, disease were evaluated using our previously reported criteria (5). In general, two investigators had to agree that the indicator lesion(s) was unequivocally smaller (regression), unchanged (stable), or larger (progression). Twenty-seven patients (57%) had measurable disease and 20 patients (43%) had evaluable disease. Patients who died or who failed to return for treatment were considered to have had tumor progression on the date of death or on the day of scheduled return, whichever occurred earlier.

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[3] Division of Medical Oncology, Mayo Clinic and Mayo Foundation, Rochester, Minn.

**Reprint requests to:* Robert T. Eagan, MD, Medical Oncology, Mayo Clinic, Rochester, Minn 55901.

TABLE 1.—Pretreatment characteristics of the 47 patients treated with MAYT

	Cell type			
	ACA	LGC	SQC	SMC
	No prior chemotherapy			
No. of evaluable patients	5	3	2	1
Median age in yrs	57	63	69	65
Range	32–61	61–63	63–75	—
Men/women	4/1	2/1	0/2	1/0
No. of patients with— ECOG PS				
0/1	0/0	0/1	0/0	0/0
2/3	3/2	2/0	1/1	0/1
Liver metastases	2	1	0	1
	Prior chemotherapy			
No. of evaluable patients	12	10	10	4
Median age in yrs	59	62	60	62
Range	45–73	49–69	50–73	54–74
Men/women	8/4	9/1	9/1	4/0
No. of patients with— ECOG PS				
0/1	1/5	1/4	0/3	1/1
2/3	2/4	3/2	5/2	0/2
Liver metastases	1	0	2	2

THERAPEUTIC RESULTS

Among 17 patients with ACA, two tumor regressions (12%) (one PR and one regression) were noted, both in patients with prior chemotherapy (*cis*-dichlorodiammineplatinum[II] in one and the combination of cyclophosphamide, adriamycin, and *cis*-dichlorodiammineplatinum[II] in the other). One response (8%) was noted among the 13 patients with LGC; this was a PR in a patient with no prior chemotherapy. Among the 12 patients with SQC, one PR (8%) was noted in a patient who had previously failed the combination of methotrexate, adriamycin, cyclophosphamide, and CCNU. No responses were seen among the five patients with SMC, four of whom had had prior chemotherapy. Twenty-one of the 47 patients (45%) had tumor progression after the first course of MAYT and therefore received only one course of treatment. In all, four responses were seen in the 47 patients for an overall response rate of 8%. Two of the four responses occurred after the first treatment with MAYT and two occurred after the second treatment. Calculated from the first day of treatment, the response durations were 53, 64, 73, and 78 days. These response durations were affected by the fact that one patient with a PR of ACA committed suicide at 64 days and another with a regression of ACA had to have his treatment stopped at 78 days after three courses because of toxic reactions.

At present, 26 of the 47 patients have died. The median survival time for all patients, estimated from a Kaplan-Meier survival curve, was 82 days (range, 4–265+ days). The estimated median survivals for patients with ACA, LGC, SQC, and SMC were 131, 98, 66, and 82 days, respectively.

TOXICITY

The most common immediate and interval toxicity is shown in table 2. Seventy-three courses of treatment were evaluable for assessment of immediate toxicity (during the 3 days of treatment). Nausea, vomiting, and diarrhea were the most frequently noted toxic effects. One patient experienced shaking chills and fever to 100°F on the third day of two consecutive chemotherapy courses. These episodes were not associated with hives or hypotension. Diphenhydramine was administered. The episodes lasted 15–30 minutes. One instance of constipation on Day 3 was noted as were two episodes of sore mouth with no associated physical findings. There were no apparent differences either in the frequency or the severity of the toxicity noted after 47 first and 26 second treatments.

Evaluable interval toxicity (toxicity occurring from Day 4 until the next scheduled treatment) was seen in 55 treatment courses (35 first and 20 second treatment courses). The incidence of severe to very severe (hospitalization required) toxicity was slightly higher (30% vs 17%) following the second course of treatment when compared to the first. Diarrhea, constipation, and neurologic abnormalities were the most common toxic effects. Hospitalization for severe constipation and/or adynamic ileus was required on three occasions. While the severe consti-

TABLE 2.—Incidences of the more common toxic effects occurring both during the 3 days of chemotherapy (immediate) and during the time period between chemotherapy courses (interval)

	% of patients	
Toxic effects	Total	With moderate to very severe toxicity*
Immediate (73 courses)		
Nausea	35	12
Vomiting	22	8
Diarrhea	42	15
Interval (55 courses)		
Diarrhea	36	20
Constipation	20	19
Neurologic	13	4
Gamma glutamyl transpeptidase elevation	14	—
SGOT elevation	13	—
Alkaline phosphatase elevation	6	—

*Moderate toxicity resulted in some significant alteration in lifestyle or function but the patient was capable of self-care, whereas with very severe toxicity the patient required hospitalization and was not capable of self-care.

pation may have represented toxicity involving the autonomic nervous system, it was arbitrarily separated from other peripheral neurotoxic effects for analysis. It is interesting that the episodes of severe constipation could not be prevented from occurring a second or third time even after dose reductions of 50%–67%. Also, the peripheral sensory neuropathy did not recover significantly after drug cessation during the lifetime of the patients in whom it occurred. The painful peripheral neuropathy affecting particularly the feet in one patient was reminiscent of the painful peripheral neuropathy occasionally seen in patients with diabetes. The neuropathy did not improve in two patients following the administration of pyridoxine.

In our phase I study, liver function test abnormalities (a ≥ 50% increase above baseline into the abnormal range in patients with no liver metastases or other cause for enzyme elevation) involving gamma glutamyl transpeptidase, SGOT, and serum alkaline phosphatase were noted. In patients for whom baseline and posttreatment determinations were available, abnormalities occurred in from 6% (alkaline phosphatase) to 14% (gamma glutamyl transpeptidase) of the patients. Overall, liver function test changes occurred after 11% of evaluable treatments. Mild and transient leukopenia occurred after four of 44 courses and thrombocytopenia after three of 44 courses. No other significant or definitely drug-related toxic effects occurred.

DISCUSSION

A phase II evaluation of MAYT was performed in patients with advanced lung cancer, the majority of whom had had prior chemotherapy and poor PS. The overall tumor regression rate was 8%. Regressions were seen in patients with ACA, LGC, and SQC, but not in patients with SMC.

Of the 47 patients treated, 21 progressed after the first course of chemotherapy. Only 26 received a second course of treatment, seven a third course, and only one a fourth course. Consequently, little can be said about the chronic toxicity. The severe constipation and unrelenting peripheral neurotoxicity seen in a minority of patients was the most troubling. We were especially concerned about our inability to prevent or ameliorate toxicity despite dose reduction or drug discontinuation. This, coupled with the 45% immediate progression rate, leads us to believe that more frequent treatment would not be more beneficial.

Our conclusion is that MAYT is an agent with some, but very limited, activity in lung cancer. Until the pharmacokinetics of the drug are better understood, we feel that further MAYT treatment of lung cancer employing the schedule described in this study is not warranted.

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Pancytopenia Induced by Aminoglutethimide in the Treatment of Breast Cancer[1]

Bellarmine Lawrence,* Richard J. Santen, Allan Lipton, Harold A. Harvey, Robert Hamilton, and Teresa Mercurio[2,3]

SUMMARY

Aminoglutethimide is an investigational agent of proven benefit in the treatment of metastatic breast carcinoma. We report herein a case of aminoglutethimide-induced pancytopenia complicated by bleeding and gram-negative septicemia. Severe pancytopenia is a rare but important side effect of this new drug and is rapidly reversible when the agent is withdrawn.

[Cancer Treat Rep 62:1581–1583, 1978]

Osler has said that "the desire to take medicine is the greatest feature which distinguishes man from his fellow creatures," but medicines are not without undesirable side effects. Aminoglutethimide originated from glutethimide by adding an amino group on the benzene ring. Introduced as an anticonvulsant in 1958, it was withdrawn from the market in 1966 due to toxicity. The principal toxicity associated with prolonged use was hypoadrenocorticism. This inhibitory effect on adrenal function has been useful in the treatment of metastatic breast cancer, metastatic prostatic cancer, and Cushing's disease (1–5). Severe pancytopenia induced by aminoglutethimide has not been previously reported.

CASE REPORT

A 71-year-old white woman had a left modified radical mastectomy on August 22, 1975, for infiltrating ductal carcinoma of the breast, and it was found that four of 21 axillary lymph nodes were positive for metastatic carcinoma. No other metastases were present at that time. Hemoglobin, hematocrit, and wbc and platelet counts were within normal limits. She was given melphalan (8 mg/day orally) for 5 days every 6 weeks as adjuvant therapy. In August 1976, a right breast biopsy revealed infiltrating ductal carcinoma. A left chest wall nodule also showed estrogen-receptor positive metastatic breast carcinoma. She was treated with diethylstilbestrol (5 mg orally three times a day) from September 3, 1976, to May 23, 1977, when the chest wall lesion progressed. On August 29, 1977, she had normal hemoglobin, hematocrit, and wbc and platelet counts, with negative bone, liver, and brain scans. On October 5, 1977, the patient began receiving aminoglutethimide therapy[4] (250 mg orally four times a day) with hydrocortisone (20 mg twice a day orally and 60 mg on retiring). The patient developed postural hypotension 1 week after aminoglutethimide therapy, and fludrocortisone (0.1 mg/day) was added. She also developed a morbilliform rash which resolved in 48 hours. On November 14, 1977, nausea, vomiting, fever, chills, and gum and rectal bleeding developed. She was transferred to the Milton S. Hershey Medical Center on November 18, 1977. She did not have any known allergy. The only other medication she received was triamterene.

Examination revealed the following: pulse, 96/minute and regular; temperature, 38.4°C; and blood pressure, 170/60 mm Hg. She appeared to be in a toxic condition and had numerous small ecchymoses and petechiae on the skin. An ear, nose, and throat examination showed petechiae on the palate. Other examinations showed the following: fundi, normal; lungs, clear; heart, S_1S_2 heard, no S_3,S_4 heard, grade II/VI systolic ejection murmur in the precordium without radiation; abdomen, normal; neurologic examination, normal; laboratory tests, wbc count $< 100/\text{mm}^3$, hemoglobin level 9.1 g/100 ml, hematocrit level 25.9%, platelet count $4000/\text{mm}^3$, BUN 19 mg/100 ml, prothrombin time 12.3 seconds, partial thromboplastin time 28 seconds, chest roentgenogram and electrocardiogram normal, haptoglobin 408

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[2] Divisions of Oncology, Endocrinology, and Hematology, Department of Medicine, The Milton S. Hershey Medical Center of the Pennsylvania State University, Hershey.

[3] We wish to thank Susan Grimes for her help in preparing this manuscript.

*_Reprint requests to:_ Bellarmine Lawrence, MD, Division of Oncology, The Milton S. Hershey Medical Center, Hershey, Pa 17033.

[4] Supplied by CIBA-Geigy Co, Summit, NJ.

mg/100 ml, and peripheral smear showed only wbcs which were lymphocytes. There were rare normal-sized platelets with normal rbc morphology. The reticulocyte count was 0.6%, the urinalysis was normal, and the total bilirubin level was 1.7 mg/100 ml. She began to receive carbenicillin and gentamicin iv. Two blood cultures grew *Pseudomonas aeruginosa* 3 days later. Aminoglutethimide was discontinued on November 18, 1977. On each of the first 4 hospital days the total wbc count was $< 100/\text{mm}^3$ and was made up entirely of lymphocytes. On the fifth hospital day the wbc count was $500/\text{mm}^3$ with 4% polymorphonuclear leukocytes and 4% metamyelocytes. Over the next 3 days the wbc count rose to a normal range characterized first by the appearance of immature forms of leukocytes. The differential wbc count became normal by the ninth hospital day. On admission, the platelet count was $4000/\text{mm}^3$. The patient required periodic transfusion of random platelets over the next 7 days. Thereafter, the platelet count rapidly rose to normal levels.

Hemoglobin was 9.1 g/100 ml on admission with a reticulocyte count of 0.6% and was 7.8 g/100 ml on the second hospital day, when the patient received a transfusion. The hemoglobin rose steadily to a level of 12.5 g/100 ml. Bone marrow examination performed on the day of admission revealed marked hypocellularity with decreased numbers of megakaryocytes and erythroid and granulocyte precursors.

After a stormy course in the hospital later complicated by myocardial infarction, the patient was discharged and was given tamoxifen (10 mg orally twice a day) and triamterene (one tablet twice a day). On February 15, 1978, cbc and platelet counts were normal except for the hemoglobin level (10.4 g/100 ml) and the hematocrit level (31.5%). On March 13, 1978, the wbc count was $5800/\text{mm}^3$, the hemoglobin level was 12.3 g/100 ml, and the platelet count was $260,000/\text{mm}^3$.

DISCUSSION

Pancytopenia has been defined as a volume of packed rbcs of < 38 ml/100 ml (altitude, 5000 ft above sea level), a total neutrophil count (polymorphonuclear leukocytes plus band cells and metamyelocytes) of $< 1800/\text{mm}^3$, a platelet count of $< 140,000/\text{mm}^3$, and a hypocellular bone marrow biopsy at some time in the course of the illness. At best, a drug, solvent, or chemical can be assumed to be toxic only by circumstantial evidence. To isolate and identify the offending agent is difficult and in some cases impossible. The diagnosis of drug-induced aplastic anemia can also be made by exclusion. In our patient, circumstantial evidence supports that the aplastic anemia was caused by aminoglutethimide. She was taking aminoglutethimide, fludrocortisone, triamterene, and hydrocortisone during the episode of aplastic anemia. Hydrocortisone and fludrocortisone are unlikely to cause aplastic anemia, and she had been taking triamterene 3 months before and 2 months after the episode, with normal hemoglobin and wbc and platelet counts. The patient started taking aminoglutethimide at a dose of 250 mg twice a day for 2 weeks during which time she did not have any symptoms of pancytopenia. As she increased the dose to 250 mg four times a day, she developed a morbilliform rash. Such transient rashes appeared to be idiosyncratic reactions in some patients taking aminoglutethimide (6,7). The drug was continued and the rash subsided without treatment in 48 hours. The patient then developed fever, chills, and a bleeding diathesis.

William et al (8) reported that *Escherichia coli* and *Pseudomonas* were the common infecting organisms in patients dying of sepsis as a result of aplastic anemia. These authors also reported that in those patients with a total neutrophil count of $> 500/\text{mm}^3$, 63% survived, whereas in those in whom the total neutrophil count decreased to $< 500/\text{mm}^3$, only 28% survived.

The sudden onset of the episode and the rapid recovery of the bone marrow 6 days after aminoglutethimide was discontinued suggest that the mechanism of pancytopenia is probably an immunologically mediated disorder which causes injury to stem cells. The only previous report of aplastic anemia in a patient receiving aminoglutethimide therapy was by Robins in 1962 (9), but that patient was taking five other anticonvulsants. In our series of 153 patients taking aminoglutethimide we have encountered only this one case of severe pancytopenia. Aplastic anemia is thus a rare but potentially fatal complication of therapy with aminoglutethimide, but is reversible after cessation of therapy.

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Antitumor Activity of Fungal Metabolites: Verrucarin β-9,10-Epoxides [1,2]

Brief Reports and Preliminary Communications

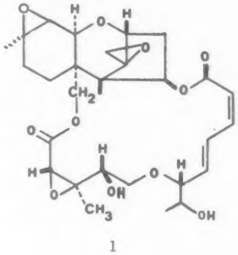
Bruce B. Jarvis,* G. Patrick Stahly, and C. R. Curtis [3]

SUMMARY

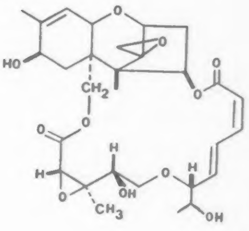
Verrucarins A (*4*) and B (*5*) and verrucarin A acetate, in vivo antileukemic inactive fungal metabolites, have been oxidized with *m*-chloroperoxybenzoic acid to give verrucarin β-9,10-epoxides which show high in vivo activity against P388 mouse leukemia.

[Cancer Treat Rep 62:1585–1586, 1978]

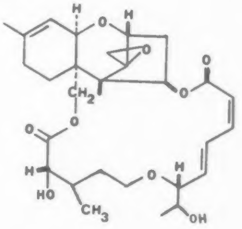
Recently, it was reported (1,2) that an extract from the Brazilian shrub *Baccharis megapotamica* yielded a series of compounds, named baccharins and baccharols, which exhibited very high in vivo activity against P388 mouse leukemia (PS).[4] The baccharins (eg, ***1***) and baccharols (eg, ***2***) are closely related to the macrocyclic sesquiterpene lactones called roridins (3) which belong to a group of mycotoxins generally known as the trichothecenes (4,5). Previously, trichothecenes have been characterized only as secondary fungal metabolites and have not been reported in vascular plants. More interesting is that although both baccharins and baccharols exhibit very high in vivo antileukemic activity, the closely related fungal roridins (eg, ***3***) are devoid of in vivo activity (1,2).



1
baccharin



2
baccharol



We report here that the oxidation of the macrocyclic trichothecenes, verrucarins A (*4*) and B (*5*),[5] with *m*-chloroperoxybenzoic acid in dichloromethane gives epoxides ***6a*** and *7*, respectively, which exhibit significant in vivo activity against PS. The structure of ***6a*** follows from its spectral data[6] and its conversion to the known acetate (6). The

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[2] Supported by the Biomedical Research Board-Public Health Service and General Research Board, University of Maryland.

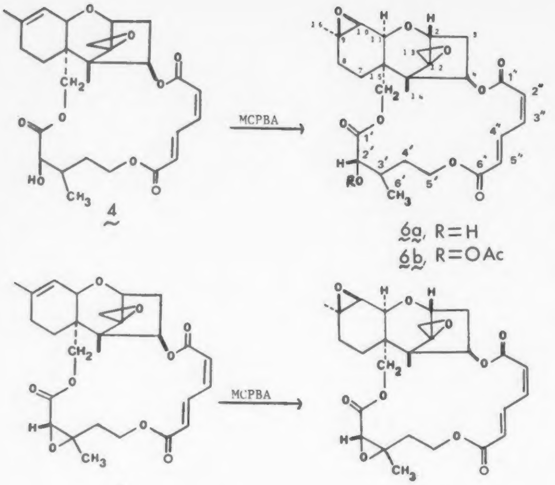
[3] Departments of Chemistry (*B. B. Jarvis* and *G. P. Stahly*) and Botany (*C. R. Curtis*), University of Maryland, College Park.

[4] The baccharins and baccharols gave typical T/C values in the range of 160–230 at dose levels of 0.5–8.0 mg/kg against PS (refs 1 and 2).

* *Reprint requests to:* Dr. Bruce B. Jarvis, Department of Chemistry, University of Maryland, College Park, Md 20742.

[5] Isolated from fermentation of *Myrothecium verrucaria* (A.T.C.C. 24571).

[6] Mp > 300°C. NMR data (CDCl$_3$, TMS at δ0): ^{1}H NMR: δ8.0 (3H, s, 14-H), 0.90 (3H, d, J=7 Hz, 6′-H), 1.41 (3H, s, 16-H), 2.04 (2H, AB, J=4 Hz, 13-H), 3.16 (1H, d, J=6 Hz, 10-H), 5.78 (1H, dd, J=4 and 8 Hz, 4-H), 6.06 (1H, d, J=16 Hz, 2″-H), 6.15 (1H, d, J=11 Hz, 5″-H), 6.70 (1H, dd, J=11 and 11 Hz, 4″-H), and 8.07 (1H, dd, J=11 and 16 Hz, 3″-H); ^{13}C NMR: δ74.8 (2-C), 34.6 (3-C), 75.2 (4-C), 49.1 (5-C), 43.6 (6-C), 22.2 (7-C), 26.0 (8-C), 57.6 (9-C), 57.0 (10-C), 67.0 (11-C), 64.6 (12-C), 47.6 (13-C), 7.3 (14-C), 62.7 (15-C), 17.1 (16-C), 174.5 (1′-C), 74.1 (2′-C), 33.2 (3′-C), 32.0 (4′-C), 61.0 (5′-C), 9.8 (6′-C), 165.1^{a} (1″-C), 127.2^{b} (2″-C), 139.0^{c} (3″-C), 138.5^{c} (4″-C), 125.3^{b} (5″-C), and 165.8^{a} (6″-C) (a–c assignments may be reversed). Acetylation of ***6a*** (acetic anhydride-pyridine) gives the known ***6b*** acetate (ref 6).



4

6a, R=H
6b, R=OAc

5

7

structure of 7 follows from its mode of synthesis and its spectral data.[7]

Although verrucarins A and B show little or no significant activity against PS,[8] the 9,10-epoxy verrucarins A and B (*6* and *7*) show marked activity in this in vivo system [antileukemic activity was assayed under the auspices of the National Cancer Institute by the procedures described by Geran et al (7)]. For ***6a***, T/C (treated/control) ranges from 210 (at 8 mg/kg) to 148 (at 0.25 mg/kg), for ***6b***, T/C ranges from 172 (at 5 mg/kg) to 145 (at 1.25 mg/kg), and for ***7***, T/C ranges from 157 (at 10 mg/kg) to 127 (at 1.25 mg/kg). All three epoxides, ***6a***, ***6b***, and ***7***, begin to exhibit toxicity at doses > 10 mg/kg in PS testing.

It is remarkable that the introduction of a single oxygen atom in the A ring of either roridins or verrucarins converts these in vivo inactive compounds to such highly active antileukemic agents. It seems to make little difference whether this additional oxygen atom is present as a 9,10-β-epoxide or an 8-β-hydroxy group.[9] What may be crucial in this system is the configuration of these oxygen groups. We are currently examining this question.

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[7] Mp 248–250°C. NMR data (CDCl$_3$, TMS at δ0): ^{1}H NMR: δ0.81 (3H, s, 14-H), 1.38 (3H, s, 16-H), 1.60 (3H, s, 6′-H), 2.48 (2H, AB, J=4 Hz, 13-H), 3.10 (1H, d, J=6 Hz, 10-H), 3.38 (1H, s, 2′-H), 5.81 (1H, dd, J-5 and 8 Hz, 4-H), 6.08 (1H, d, J=16 Hz, 2″-H), 6.16 (1H, d, J=11 Hz, 5″-H), 6.66 (1H, dd, J=11 and 11 Hz, 4″-H), and 7.90 (1H, dd, J=11 and 16 Hz, 3″-H); ^{13}C NMR: δ78.5 (2-C), 34.7 (3-C), 75.3 (4-C), 48.9 (5-C), 43.3 (6-C), 22.2 (7-C), 26.2 (8-C), 57.6 (9-C), 57.1 (10-C), 67.4 (11-C), 64.7 (12-C), 47.8 (13-C), 7.6 (14-C), 63.2 (15-C), 17.2 (16-C), 167.6 (1′-C), 58.5 (2′-C), 61.8 (3′-C), 37.1 (4′-C), 60.8 (5′-C), 15.7 (6′-C), 166.0^a (1″-C), 127.6^b (2″-C), 138.8^c (3″-C), 138.0^c (4″-C), 125.5^b (5″-C), and 165.1 (6″-C) (a–c assignments may be reversed).

[8] Verrucarin A and verrucarin A acetate exhibit toxicity at doses > 2 mg/kg in PS testing. In this test for these compounds at doses below this level, T/C values are all < 130. Verrucarin B has a T/C of 142 at 3.0 mg/kg and a T/C of < 130 at ≤ 1.0 mg/kg in PS testing.

[9] We are investigating whether the 8-β-hydroxy verrucarins will also prove to be active against in vivo tumors.

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Levamisole Granulocytopenia in Patients Receiving an Adjuvant Chemoimmunotherapy Program After Surgery for Breast Carcinoma With Axillary Lymph Node Involvement[1,2,3]

Brief Reports and Preliminary Communications

C. L. Vogel,* D. L. Lipscomb, M. A. Silverman, A. L. Kerns, P. W. Mansell, and E. V. Sugarbaker[4,5]

Patients with primary breast carcinoma with axillary lymph node metastases have a high risk of relapse after standard or modified radical mastectomy (1). Recent updates of the use of adjuvant postoperative chemotherapy in this clinical situation show continuing benefit only in premenopausal and not in postmenopausal women (2,3). The anthelmintic, laevotetramisole (levamisole), has been shown to have immunorestorative properties (4) and there are reports of possible benefit using this drug as a clinical "immunotherapeutic" agent in lung cancer (5), in metastatic breast cancer (6), and as an adjunct to radiation therapy for locally advanced breast cancer (7). Since cytotoxic chemotherapy is immunosuppressive and since such immunosuppression theoretically could interfere with host immune responses against a tumor, a clinical trial was designed using a short (6-month) course of chemotherapy coupled with levamisole "immunorestorative" therapy. A preliminary analysis of the toxicity of this chemoimmunotherapy regimen forms the basis of this report.

PATIENTS AND METHODS

Between September 1976 and December 1977 a total of 34 women with newly diagnosed primary breast cancer and axillary lymph node metastases were treated after mastectomy with 6 months of combination chemotherapy and levamisole immunotherapy. At the conclusion of the initial 6-month chemoimmunotherapy program, levamisole was to be continued for an additional 6 months with no further cytotoxic chemotherapy being given. A prechemotherapy evaluation included a cbc count, liver function tests, and appropriate tests to assure lack of residual disease. Initially, cbc counts were done weekly during the first two courses of chemotherapy and monthly thereafter. Midway into the study, cbc counts were done weekly for 10 weeks after patients had completed chemotherapy and were only receiving levamisole immunotherapy; after that 10-week period, blood cell counts were done monthly.

In 25 of these patients, chemotherapy was given according to the doses and schedule previously described by Bonadonna and co-workers (3). Nine patients received a modification of the chemotherapy protocol as described in table 1. Levamisole (in both chemoimmunotherapy programs) was given at a dose of 90 mg/m^2 divided into two or three separate doses each day on the same 2 successive days each week, both during the 6 months of chemotherapy and during the 6 months after completion of the chemotherapy.

RESULTS

Of the 34 patients studied, three were removed from the study within 3 months after the start of treatment for reasons unrelated to possible levamisole toxicity. Of the remaining 31 patients, 14 (45%) had some symptoms clearly attributable to levamisole including dizziness, nausea, metallic taste, flu-like syndrome, skin rash, and granulocytopenia (< 1500 cells/mm^3). Six of these 31 patients were removed from the study early because of either protocol noncompliance or patient refusal secondary to chemotherapy toxicity. Of the remaining 25 pa-

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[3] Levamisole tablets were kindly supplied by Janssen R and D, Inc, New Brunswick, NJ.

[4] Divisions of Breast Cancer, Medical Oncology, and Surgical Oncology, Department of Oncology, University of Miami School of Medicine, and the Comprehensive Cancer Center for the State of Florida, Miami.

[5] We thank Drs. Mark Saltzman, Francisco Tejada, Michael Troner, and Arnold Blaustein who cared for some of the study patients.

**Reprint requests to:* Charles L. Vogel, MD, Division of Breast Cancer, University of Miami School of Medicine, PO Box 016960, Miami, Fla 33101.

TABLE 1.—Modified chemotherapy regimen (9 patients)

Drug	Dose* (mg/m²)	Drug administration					
		Month					
		0	1	2	3	4	5
Cyclophosphamide	500	×	×	×	×	×	×
5-Fluorouracil	500	×	×	×	×	×	×
Adriamycin	50		×		×		×
Methotrexate	40	×		×		×	

* All drugs given by iv push on Day 1 of a given course.

tients, one was removed from the study because of a skin rash which only resolved with discontinuation of levamisole; six (24%) patients were removed from the study because of granulocytopenia apparently related to levamisole administration.

Each of the six patients with granulocytopenia developed this complication within 4–8 weeks after the last dose of their sixth course of cytotoxic chemotherapy and each patient had at least one normal interval granulocyte count between the end of chemotherapy and the development of granulocytopenia. The lowest absolute granulocyte counts recorded for these patients were 0, 0, 4, 540, 759, and 926/mm³. Three patients had to be hospitalized because of proved or suspected sepsis but all recovered with antibiotic therapy. The other three patients remained asymptomatic throughout the study. Three of the patients had an associated modest decrease in hemoglobin concentration and two had associated decreases in platelet counts. Bone marrow aspirates done in the three hospitalized patients were hypocellular with a virtual absence of granulocyte precursors. Peripheral granulocyte count recovery in five of the six patients was prompt (7–10 days) after discontinuation of levamisole. The sixth patient remains granulocytopenic 2 months after discontinuation of levamisole and there is now some question whether her granulocytopenia is levamisole-related.

DISCUSSION

Six of 25 (24%) women developed granulocytopenia during treatment with levamisole used as an adjunct to combination chemotherapy after mastectomy for primary breast cancer. Granulocytopenia had only been reported as a complication of levamisole treatment in 23 of 1619 (1.4%) patients from 77 clinical trials in which the presence or absence of adverse effects was reported (8). Among several published studies of the use of levamisole specifically in cancer there have been only six of 406 (1.5%) cases of granulocytopenia associated with levamisole (8–15). Five additional patients with this complication have been reported recently (16).

The mechanism of the granulocytopenia secondary to levamisole is not clear although most of the available evidence so far (10) points to an immunologic explanation, leukoagglutinins having been found in a number of cases. Although there may be many factors associated with the formation of leukoagglutinins, particularly in patients with complex disease processes, it is tempting to speculate on the possible part that prior chemotherapy may have played. Chemotherapy is immunosuppressive and the possibility exists, therefore, that any immunologic reaction, such as the formation of leukoagglutinins, would be suppressed during the administration of chemotherapy. Immunologic reactivity has been shown to rebound following chemotherapy with a variety of agents, for instance, methotrexate (17) and cyclophosphamide (18), which are used in the cyclophosphamide, methotrexate, and 5-fluorouracil combination. It is particularly noteworthy that antimetabolites such as methotrexate and possibly 5-fluorouracil are capable of suppressing the primary immune response, such as the formation of a leukoagglutinin (19). Once the suppressive therapy is stopped, there is a return of immunologic activity. As a result of immunologic rebound, the ability to form leukoagglutinins would also return, possibly at a much higher titer than if no immunosuppressive agents had been given before. Detailed immunologic and leukoagglutinin studies are currently underway in patients being treated with this adjuvant chemoimmunotherapy program, which may enable us to answer this question. There seems to be little possibility that this phenomenon is solely dose-related.

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Ophthalmologic Toxicity After *cis*-Dichlorodiammineplatinum(II) Therapy [1]

Stanley Ostrow,[2,*] Davis Hahn,[2] Peter H. Wiernik,[2] and Richard D. Richards[3]

SUMMARY

Two cases of apparent ophthalmologic toxicity which occurred during treatment with *cis*-dichlorodiammineplatinum(II) (DDP) are described. In the first case, a patient with testicular carcinoma developed papilledema after three courses of DDP and adriamycin therapy. In the second case, a patient with breast cancer developed retrobulbar neuritis after three courses of DDP therapy. The differential diagnosis and possible explanation for these toxic effects are discussed.

[Cancer Treat Rep 62:1591–1594, 1978]

cis-Dichlorodiammineplatinum(II) (DDP), an investigational chemotherapeutic agent, is being studied in patients with a wide variety of tumors. The most common toxic effects observed to date have included nephrotoxic and ototoxic manifestations, gastrointestinal disturbances, and bone marrow suppression (1). Less frequently, seizures and peripheral neuropathy have also been reported (2,3). We have noted apparent ophthalmologic toxicity occurring in two patients receiving DDP and describe this new observation.

Patient 1

A 53-year-old male underwent an orchiectomy for embryonal cell carcinoma in April 1977. A physical examination including neurologic testing was unremarkable. A chest roentgenogram revealed bilateral pulmonary nodules, and an iv pyelogram (IVP) showed partial obstruction of a proximal ureter. In June 1977 he began to receive DDP therapy at a dose of 60 mg/m^2 on the first and eighth day of treatment along with adriamycin at a dose of 40 mg/m^2 on the first day of treatment. Treatment courses were repeated every 3 weeks. The patient was hydrated prior to DDP therapy and mannitol diuresis was given during the infusion.

A complete tumor response was evident after three treatment courses. This was documented by a return of the IVP and chest roentgenogram to normal and no evidence of primary or metastatic disease as shown by physical examination, radiographs, and laboratory data. A total dose of 150 mg/m^2 of DDP had been given in the first three courses. Two weeks after his third course of treatment, the patient developed malaise, nausea, vomiting, and frontal headaches. One week later he was noted to have blurred disc margins on funduscopic examination. An ophthalmologic consultant found papilledema of two diopters, but no evidence of central scotomata or decrease in visual acuity. A computerized axial tomogram of the head (CAT scan) was normal. A fourth course of DDP and adriamycin was then begun. A lumbar puncture performed 1 day after the first DDP dose in the fourth course revealed the following: opening pressure, 280 mm H_2O; closing pressure, 210 mm H_2O; protein, 63 mg/100 ml; and glucose, 54 mg/100 ml. A differential cell count revealed only eight mononuclear cells, and cultures were negative. A neurologic examination revealed diminished position, vibratory, and tactile sensation in both lower extremities. The patient was given 4 mg of dexamethasone every 6 hours orally. Forty-eight hours later, his headache had improved and the papilledema was diminished. A repeat lumbar puncture revealed the following: opening pressure, 130 mm H_2O; 2 monocytes/mm^3, protein, 81 mg/100 ml; and glucose, 94 mg/100 ml. Dexamethasone was tapered and discontinued. Repeated neurologic examinations including funduscopic examination have revealed resolution of the papilledema. Lumbar punctures and CAT scans performed 2 months after DDP was stopped have been unremarkable. The patient remained in remission and was receiving alternate chemother-

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[2] Baltimore Cancer Research Program, Division of Cancer Treatment, National Cancer Institute, Md.

[3] Department of Ophthalmology, University of Maryland Hospital, Baltimore.

**Reprint requests to:* Stanley Ostrow, MD, Baltimore Cancer Research Program, Division of Cancer Treatment, National Cancer Institute, University of Maryland Hospital, 22 S Greene St, Baltimore, Md 21201.

apy for 5 months, but has recently relapsed with lung metastases.

Patient 2

In 1972 a 67-year-old female underwent a left mastectomy for carcinoma of the breast. She remained free of disease until 1977 when she developed biopsy-proven lung metastasis and multiple areas of metastatic disease to the brain documented by CAT scan.

She received 5000 rads of whole brain irradiation over 6 weeks with a return of the CAT scan to normal 2 months after therapy was discontinued. She was then given cyclophosphamide, methotrexate, and 5-fluorouracil (5-FU) and intradermal methanol extracted residue of bacillus Calmette-Guérin. She developed progressive lung disease after 11 courses of therapy, and beginning in August 1977 she received three courses of adriamycin and vincristine every 3 weeks without response. Beginning in November 1977 she received tamoxifen for 2 months with no response; the tamoxifen was discontinued. Beginning in January 1978 she was given 100 mg/m^2 of DDP iv every month and received three courses of therapy with stabilization of her lung disease.

The patient has been followed by the same ophthalmologist since 1961 when she was noted to have a dense cataract in her left eye and 20/20 vision with corrective lenses in her right eye. Since that time, there has been no change in visual acuity in the right eye. Two weeks after her third course of DDP she complained of decreased vision in her right eye. A funduscopic examination of the right eye revealed no evidence of cataract formation, normal vessels, and no evidence of hemorrhage or papilledema. The patient was unable to see fingers placed 6 inches in front of her right eye. A diagnosis of retrobulbar neuritis was made. A CAT scan and spinal fluid cytology revealed no evidence of metastatic disease. The serum zinc level was 47 μg/dl (normal, 69–133 μg/dl by atomic absorption photometry). Zinc gluconate at a dose of 30 mg/day was administered with a return of the serum zinc to the normal range. DDP was discontinued for 2 months without visual improvement. However, because of progressive disease occurring when DDP was discontinued, therapy has been re-instituted.

DISCUSSION

Patient 1, who had embryonal cell carcinoma of the testes, received four courses of DDP and adriamycin therapy every 3 weeks with complete resolution of measurable metastatic disease. Because of a previously unreported neurotoxic effect (papilledema), therapy was discontinued and alternate therapy was given. Patient 2, who had metastatic breast carcinoma, developed retrobulbar neuritis after three courses of DDP therapy alone.

Both papilledema and retrobulbar neuritis have been reported in a number of systemic diseases and after treatment with a number of drugs (4–11).

Among the heavy metals which have been reported to cause papilledema or retrobulbar neuritis are gold, mercury, lead, and thallium (6,7,10). In lead poisoning, the optic nerves may be injured by increased intracranial pressure or by a direct toxic action of lead on the nerve. Papilledema is thought to be secondary to increased intracranial pressure (due to encephalopathy) (6,12) where it is seen in a minority of cases (13) and is also thought to accompany retrobulbar or optic neuritis (6,12). The mechanism of this neuritis is not known (6). In thallium poisoning, the most common eye disturbance has been optic neuritis, with the retrobulbar portion being most often affected. In one case of thallium poisoning, anatomic study of the eye revealed a highly selective incomplete loss of retinal ganglion cells with the nerve fibers and optic nerves almost completely atrophic. The anterior portion of the eye and the rods and cones appeared to be undamaged (14,15).

Pharmacokinetic studies of radioactive DDP after a single iv administration suggest poor penetration into the central nervous system (16,17). Pharmacokinetic studies of DDP following a 6-hour infusion suggest plasma accumulation 3–4 days after injection. In one patient, 2.5% of serum levels appeared in cerebrospinal fluid during the first dose and 5% during the second dose, suggesting that accumulation may occur (7). It is possible that repeated doses of DDP can lead to accumulation in the central nervous system and result in neurotoxicity.

The question arises as to whether there were any other possible causes of papilledema or retrobulbar neuritis in our patients reported here. The first patient did not have any of the known systemic causes of papilledema and was not taking any medication known to cause papilledema. He had no evidence of metastatic disease in the central nervous system.

Adriamycin, an anthracycline antibiotic, which was given with DDP to the first patient, has not been reported to cause papilledema or optic neuritis. The only ophthalmologic toxic reaction reported in patients receiving adriamycin consists of lacrimation seen within 15 minutes after drug administration (18). Central nervous system toxicity (seizures) has been reported following adriamycin given as a single agent in a few patients within 48 hours of administration (19,20). Most patients had evidence of central nervous system metastases. Adriamycin does not appear to significantly accumulate in the brain in animal studies[4] and does not enter the spinal fluid in man (21). These points would appear to exclude this drug as a contributing cause of papilledema.

Retrobulbar neuritis can occur in patients with carcinomatous meningitis (not observed in our second patient) and in patients with malignant disease without direct involvement of the visual pathways by tumor cells. In one reported case there was pathologically verified bilateral demyelinating disease of the optic nerves in a patient with bronchogenic carcinoma (22). The mechanism by which this occurs is unknown although vitamin deficiency or malnutrition has been suggested (22). In our patient, the retrobulbar neuritis developed at a time when there was no evidence of cerebral metastases and when the lung disease had stabilized. During the time when she had cerebral metastases and progressive lung disease, there was no evidence of retrobulbar neuritis. This patient also had a low serum zinc level drawn after the onset of retrobulbar neuritis. No serum zinc levels were obtained prior to DDP therapy. The cause of the low serum zinc is unknown but low levels have been seen in patients with bronchogenic carcinoma and Hodgkin's disease (23,24). There are reports of low serum zinc seen in patients who had retrobulbar neuritis secondary to ethambutol or alcohol (25). To our knowledge, there have been no reports of zinc deficiency per se as a cause of retrobulbar neuritis, although it has been suggested that zinc deficiency should be looked for in certain patients with optic neuropathy (26). Our patient was given supplemental zinc gluconate with serum levels rising to normal without improvement in visual symptoms.

In the absence of known causes of retrobulbar neuritis, the use of DDP, a heavy metal in the same family of elements known to cause retrobulbar neuritis, is the most likely cause in our second patient.

These two cases suggest probable DDP ophthalmologic toxicity. With increasing use of DDP chemotherapy, more cases of ophthalmologic toxicity may be reported in the future. Further studies are needed to elucidate the mechanism of these ophthalmologic toxic effects so that prevention may be intelligently approached.

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Cyclophosphamide, Adriamycin, Methotrexate, and Procarbazine (CAMP) Treatment of Non-Oat Cell Bronchogenic Carcinoma[1]

Nicholas J. Vogelzang, Philip D. Bonomi,* Arthur H. Rossof, and Janet Wolter[2]

The efficacy of chemotherapy for metastatic non-oat cell bronchogenic carcinoma (MNOBC) has been disappointing. Extensive trials have documented very low response rates for single-agent therapy (1). Combination chemotherapy has shown moderate activity with occasional prolongation of survival (2). A recent report of a 48% response rate in MNOBC using cyclophosphamide, adriamycin, methotrexate, and procarbazine (CAMP) (3) prompted our investigation of this combination. The purpose of this paper is to report our experience with the CAMP regimen in MNOBC.

METHODS AND MATERIALS

From September 1976 to December 1977, 32 patients with stage III-M_1 MNOBC (4) were treated with CAMP at the Rush-Presbyterian-St. Luke's Medical Center. Patient selection criteria included no prior chemotherapy, measurable biopsy-proven bronchogenic carcinoma, Eastern Cooperative Oncology Group (ECOG) performance status (PS) of ≤ 3, wbc and platelet counts > 4000 and $100{,}000/\mu l$ respectively, and creatinine and bilirubin levels < 1.5 and 2.0 mg/dl respectively. There could be no congestive heart failure or recent myocardial infarction. Radiated lesions were considered nonmeasurable. There was no attempt to systematically irradiate the primary lung lesions.

The CAMP regimen consisted of cyclophosphamide (300 mg/m^2 iv), adriamycin (20 mg/m^2 iv), methotrexate (15 mg/m^2 iv), and procarbazine (100 mg/m^2 orally) daily. The iv drugs were given on Days 1 and 8 and procarbazine was given on Days 1–10 of a 28-day cycle. Dose modifications were based on wbc and platelet counts at the start of each cycle. Fifty percent of the drug was given when the wbc count was between 3000 and $4000/\mu l$ or the platelet count was between 75,000 and $100{,}000/\mu l$. In the case of lower counts, treatment was withheld until the wbc count reached $3000/\mu l$ and the platelet count reached $100{,}000/\mu l$. Adriamycin was discontinued when the total dose of 450 mg/m^2 of body surface area was reached.

RESPONSE DEFINITIONS

Tumor responses were defined according to ECOG criteria. Complete response (CR) was defined as the disappearance of all clinically detectable disease. Partial response (PR) was defined as a reduction of $\geq 50\%$ of the sum of the products of the perpendicular diameters of measurable lesions, without progression of any lesion or the appearance of any new lesion, for ≥ 2 months. Stable disease (SD) was defined as $< 50\%$ reduction in the measurable area of disease with no new metastases. Progressive disease (PD) was defined as the appearance of new metastases or a $> 25\%$ increase in pre-existing lesions.

Study Parameters

Measurement of lesions, chest radiographs, and cbc and platelet counts were done each month. Blood chemistries and isotope scans were done at 2–3-month intervals.

RESULTS

Thirty-two patients were entered in the study: 13 with adenocarcinoma, ten with squamous cell carcinoma, and nine with undifferentiated large cell carcinoma. Twenty-six patients were evaluable for response. They received from one to 14 courses of CAMP with a median of five courses.

CR and PR were seen in eight of 26 patients (31%). There were an additional eight patients with SD (31%). The response rate by histologic type is listed in table 1.

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[2] Section of Medical Oncology, Department of Internal Medicine, Rush-Presbyterian-St. Luke's Medical Center, Chicago, Ill.

* *Reprint requests to:* Philip D. Bonomi, MD, Section of Medical Oncology, Department of Internal Medicine, Rush-Presbyterian-St. Luke's Medical Center, 1753 W Congress Pkwy, Chicago, Ill 60612.

TABLE 1.—Response rate by histologic type

Histologic type	No. of patients	No. of responses CR	PR	SD	CR + PR (%)
Adenocarcinoma	12	1	4	5	42
Squamous cell carcinoma	7	0	1	1	14
Large cell undifferentiated carcinoma	7	0	2	2	28
Totals	26	1	7	8	31

PS clearly correlated with response. Of the 16 patients with CR, PR, and SD, 14 had an ECOG PS of 0 or 1. Of the nine nonresponders, five had a PS of 2. No patients with a PS of 3 or 4 were entered in the study.

Toxicity

There was little hematologic toxicity. Leukopenia (< 3000 cells/μl) occurred in four patients, one of whom was receiving radiotherapy concomitantly. Nadir counts were not routinely obtained. A platelet count $< 90{,}000/\mu$l occurred once. One patient who had received two courses of radiotherapy to the mediastinum experienced life-threatening recall esophagitis. Gastrointestinal toxicity was significant, and was apparently related to the procarbazine. Doses of this drug were reduced in five patients. In an additional seven patients, the drug was stopped because of nausea and vomiting.

Survival

The median survival of the ten nonresponders was 4.5 months. The median survival of the eight patients with SD is currently 7+ months with five of the eight still living. The patients who have shown an objective remission (CR or PR) have a current median survival of 9+ months with five of eight patients still living. There is a statistically significant difference in survival between patients who experience CR, PR, or SD and nonresponding patients ($P < 0.005$, Student's t test). Likewise, the difference in survival between patients with SD only and nonresponding patients is significant ($P < 0.005$, Student's t test). A survival curve depicting these data, derived by the life-table method, is shown in figure 1. Of 16 patients in the CR, PR, and SD group, five had central nervous system (CNS) metastases as the only expression of PD. Of ten patients with PD, one had CNS metastases as the initial expression of disease progression.

DISCUSSION

Single-agent chemotherapy produces low response rates and does not prolong survival in MNOBC (1,2). In contrast, increased response rates and prolongation of survival have been observed with several combination regimens (5,6). The originators of the CAMP regimen, Bitran et al (7), have reported a 33% response rate with median survivals of 12.6 months in responders versus 2.4 months in nonresponders ($P < 0.001$).

Our response rate of 31% and the 9+-month median survival in responders versus 4.5 months in nonresponders confirm the activity of the CAMP regimen. A majority of the patients studied by Bitran and associates received thoracic radiation to a symptomatic pulmonary primary immediately prior to CAMP, and these investigators suggested that radiation therapy might play a role in the increased response and prolongation of survival. None of our eight responding patients received irradiation immediately before CAMP, and only one responding patient received radiotherapy during CAMP. It therefore appears that, in our patients, CAMP alone improved survival. However, a randomized prospective trial will be required to determine if there is synergism between CAMP and radiation.

In our study, 42% of the patients with adenocarcinoma responded. Hansen et al (5) using cyclophosphamide, methotrexate, and CCNU showed a 38% response rate in adenocarcinoma as compared to a 6% response rate in squamous cell carcinoma and a 10% response rate in large cell carcinoma. Edmonson et al (8) have shown that objective disease stability using cyclophosphamide and CCNU is associated with prolonged survival in patients with adenocarcinoma. We have made similar observations in patients with adenocarcinoma. The apparent greater sensitivity of adenocarcinoma to chemotherapy and especially to CAMP should be further investigated.

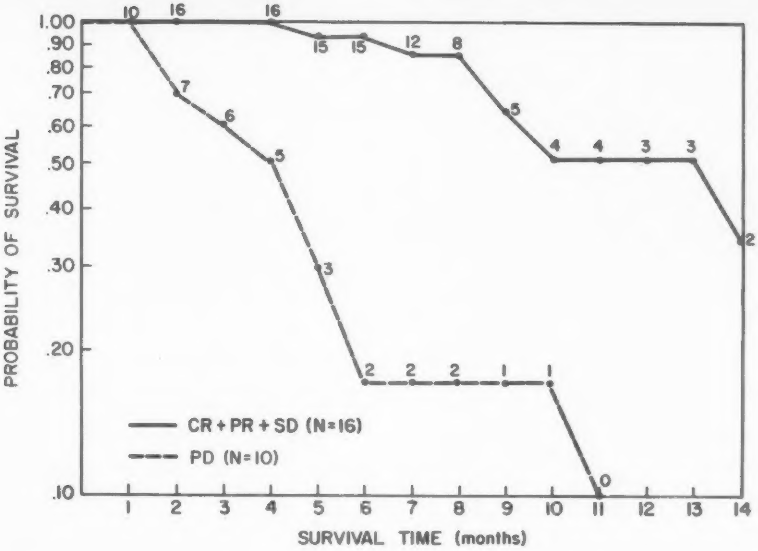

FIGURE 1.—Survival curve showing the difference between patients with PD and responding patients (life-table method).

Although procarbazine can cross into the cerebrospinal fluid (9), there were five patients who relapsed in the CNS, while other sites of disease were controlled. All five patients had at least three full courses of procarbazine. This suggests the failure of procarbazine to control CNS metastases. Since procarbazine was poorly tolerated in our patients, perhaps another CNS-active agent could be substituted.

In conclusion, CAMP is effective chemotherapy for MNOBC, especially of the adenocarcinoma type. The toxicity of this regimen is mild. CAMP should be included in cooperative group trials.

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Clinical Trial of Rubidazone in Advanced Squamous Cell Carcinoma of the Lung and Adenocarcinoma of the Large Intestine [1,2]

Roberto J. Fraile,* Michael K. Samson, Thomas R. Buroker, Robert O'Bryan, Laurence H. Baker, and V. K. Vaitkevicius [3,4]

SUMMARY

Sixteen patients with disseminated squamous cell carcinoma of the lung and 26 patients with adenocarcinoma of the colon and rectum were given rubidazone. Only one partial remission was observed in a previously untreated patient who had local recurrence of a rectal adenocarcinoma. The main toxic effects observed in previously treated patients consisted of leukopenia and thrombocytopenia. Also observed were anorexia, nausea, vomiting, alopecia, fever, and chills. Cardiotoxicity was observed in one patient after a total dose of 720 mg/m^2 of rubidazone. It is concluded that rubidazone is a relatively inactive compound in the management of these two diseases.

[Cancer Treat Rep 62:1599–1601, 1978]

The chemotherapy for advanced squamous cell cancer of the lung and adenocarcinoma of the colon and rectum has remained ineffective despite advances in therapy for other solid tumors. The results obtained with both single-agent and combination chemotherapy have shown limited effectiveness in both tumor systems and clinical trials with new potentially active agents would appear to be indicated. While adriamycin has some reported activity against squamous cell carcinoma of the lung (1–3), it is inactive in colorectal carcinoma (4). When evaluating analogs, it is important to test the new drug in the analog-sensitive and -insensitive human cancers.

Rubidazone is a semisynthetic derivative of daunorubicin which exerts its antitumor activity by intercalating DNA and inhibiting DNA and RNA synthesis and possibly by in vivo release of daunorubicin (5). This drug is active in numerous animal tumors with a spectrum similar to that of adriamycin and daunorubicin but superior to daunorubicin in P388 leukemia, ip B16 melanoma, and Lewis lung carcinoma (5). In clinical trials, rubidazone has shown activity in acute leukemias and possible activity in lymphomas (6,7). The drug appears to be well-tolerated, although alopecia, skin rash, nausea, vomiting, mucositis, diarrhea, fever, chills, and myelosuppression have commonly been observed (6,7). Congestive heart failure has also been observed in patients previously treated with anthracyclines. Additionally, renal and liver dysfunction have occasionally been noted.

These data provided the impetus for a phase II trial of rubidazone for patients with incurable squamous cell cancer of the lung or adenocarcinoma of the large intestine.

MATERIALS AND METHODS

The criteria for patient eligibility included the following: written consent concerning the investigational nature of the study; life expectancy of at least 6 weeks; presence of measurable disease outside a concurrently or previously irradiated site; no chemotherapy or radiotherapy for 3 weeks prior to starting rubidazone; an initial wbc count of $\geq 3000/\text{mm}^3$ and platelet count of $\geq 100{,}000/\text{mm}^3$; adequate renal function defined as a serum creatinine ≤ 2.0 mg/100 ml and BUN ≤ 4.0 mg/100 ml; and adequate liver function defined as a serum bilirubin ≤ 2.0 mg/100 ml. Patients with a history of previous therapy with anthracycline derivatives and those with significantly abnormal electrocardiograms and/or

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[3] Department of Oncology, Wayne State University School of Medicine, and Division of Oncology, Henry Ford Hospital, Detroit, Mich.

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Reprint requests to: Roberto J. Fraile, MD, Department of Oncology, Harper-Grace Hospitals, 3990 John R St, Detroit, Mich 48201.

prominent cardiac disease were excluded from the study.

Rubidazone was given after it was reconstituted with a diluent, further diluted in 250 ml of 5% dextrose in water, and infused over 30–60 minutes with care to avoid extravasation. Patients were premedicated with 50 mg of diphenhydramine given iv to prevent the fever, chills, and hives that were observed in the first patients treated with this drug. Good-risk patients received an initial dose of 150 mg/m^2 of rubidazone, while poor-risk patients, defined as those with prior *extensive* radiation therapy and/or *extensive* chemotherapy or $>$ 65 years of age, received an initial dose of 120 mg/m^2. The dose was reduced by 50% if the serum bilirubin was between 1.2 and 2.0 mg/100 ml.

Rubidazone was given at 3-week intervals provided there was sufficient evidence of recovery from the nadir leukocyte and platelet counts observed with the previous course of therapy (wbcs $\geq$ 3000/mm^3 and platelets $\geq$ 100,000/mm^3); subsequent doses were adjusted to nadir blood cell counts and patient tolerance.

Patients were evaluated by serial cbc and platelet counts, liver and renal function tests, appropriate roentgenograms and isotope studies, tumor measurements, electrocardiograms, measurements of systolic time intervals, and echocardiograms. The drug was to be discontinued after 3500 mg/m^2 (3000 mg/m^2 if the patient had received cardiac irradiation), or if the patient developed cardiac toxicity. A course of therapy was defined as 1 day of drug therapy followed by 20 days of no therapy, and a minimum of two courses was required for an adequate trial.

Complete remission was defined as a disappearance of all clinical evidence of active tumor for a minimum of 4 weeks together with a disappearance of all tumor-related symptoms. Partial remission (PR) was defined as a $>$ 50% decrease in the sum of the products of the largest perpendicular diameters of all measured lesions lasting for at least 4 weeks in the absence of a significant increase in any one lesion or the appearance of new lesions. Progressive disease was defined as an unequivocal increase of any measured lesion or the appearance of new lesions.

PATIENT SELECTION

Lung Cancer

Sixteen patients with squamous cell carcinoma of the lung, 13 men and three women (nine of whom were considered poor-risk patients), were entered in this study. The median age was 59 years (range, 36–73 years) and the median Karnofsky performance status was 55 (range, 50–90). All patients had disseminated disease with metastasis to the lymph nodes (five), liver (four), brain (three), bone (seven), and mediastinum (one). Seven patients had received previous chemotherapy and had clearly progressive disease at the time of entrance into the study. Twelve patients had been previously treated with radiation therapy.

Colorectal Cancer

Twenty-six patients with adenocarcinoma of the large intestine, 16 men and nine women (16 of whom were considered poor-risk patients), were entered in this study and 25 of these patients were evaluable for response and toxicity. One patient was lost to followup. The median age was 68 years (range, 43–75 years) and the median Karnofsky performance status was 80 (range, 40–100). All but one of these patients had disseminated disease with metastasis to the liver (19), bone (five), lung (ten), lymph nodes (four), and skin (two); two patients had intra-abdominal masses. One patient had locally recurrent, unresectable disease.

Eighteen of these patients had received prior chemotherapy with 5-fluorouracil alone or in combination with other agents; five had received prior radiation therapy.

RESULTS

There was no response observed in the 16 patients with squamous cell carcinoma of the lung. In the 26 patients with colorectal adenocarcinoma, there was one PR (4%) with a response duration of 15 weeks. This response occurred in a patient who had a perineal recurrence and had not received any prior chemotherapy.

Toxicity

Major nonhematologic toxic effects consisted of anorexia (28%), nausea (40%), vomiting (17%), alopecia (15%), and fever and chills (5%). A local skin slough was observed in one patient due to extravasation of the drug.

There was no acute cardiac toxicity observed, but one patient with squamous cell carcinoma of the lung, previously treated with radiation therapy to the mediastinum and left hilar area and a history of arteriosclerotic heart disease, developed symptoms and signs of congestive heart failure after a total dose of 720 mg/m^2 of rubidazone had been administered. Rubidazone cardiotoxicity cannot be

TABLE 1.—Hematologic toxicity of rubidazone

Dose (mg/m^2)	No. of patients	Wbc count			Platelet count		
		Median nadir (× 10^3/mm^3)	Range	Median days to nadir	Median nadir (× 10^3/mm^3)	Range	Median days to nadir*
Patients with prior chemotherapy							
120	10	4.7	1.2–13.3	17	200	50–500	22
150	9	3.1	1.6–30.0	20	193	5–465	16
Patients with no prior chemotherapy							
120	8	4.9	3.1–13.1	—	242	160–465	—
150	5	4.9	0.5–7.9	13	306	58–347	11

*Calculated only for patients with wbcs < 3000/mm^3 and platelets < 100,000/mm^3.

excluded since a consent for autopsy was denied. No liver or renal toxicity was observed.

Hematologic toxicity and days to nadir for dose levels of 120 and 150 mg/m^2 are outlined in table 1. As can be seen, hematologic toxicity was observed mainly in previously treated patients, with significant leukopenia (< 3000 cells/mm^3) occurring in 54% (28 of 52 courses) and thrombocytopenia (< 100,000 cells/mm^3) in 13% (seven of 52 courses), while in untreated patients this toxicity occurred in only 33% (11 of 33 courses) and 6% (two of 33 courses), respectively. There did not appear to be any cumulative bone marrow suppression or any inordinate delay in administering subsequent courses of therapy.

In general, toxicity was acceptable and manageable. However, there was one drug death due to cerebral hemorrhage following the administration of a full dose of the drug (150 mg/m^2) in a patient who had a pretreatment serum bilirubin value of 2 mg/100 ml. At the time of his death, 9 days after the first dose of rubidazone, the wbc count was 500/mm^3 and the platelet count was 5000/mm^3.

DISCUSSION

An every-3-week schedule for rubidazone was chosen in this phase II study, based in part on the known pharmacokinetics as well as on the previous clinical experience with the parent compounds daunorubicin and adriamycin. This schedule proved to be practical and well-tolerated, with toxicity essentially predictable and manageable. The need for dose reduction in the presence of liver dysfunction and for close cardiovascular observation is stressed.

We observed one PR in a patient with rectal cancer and none in the patients with squamous cell cancer of the lung. These results, obtained in patients previously treated or untreated and with a good- or poor-risk performance status, are similar to those results of the previously evaluated anthracycline, adriamycin. The dose of rubidazone received by untreated patients may have been suboptimal since only one third of them experienced moderate to severe myelotoxicity. It is possible, but unlikely, that these traditionally anthracycline-resistant tumors might have responded to a higher dose. While our protocol called for a dose escalation by 30 mg/m^2 when no toxicity was observed, this was frequently not possible since patients failed to respond to rubidazone and were taken off the study. We conclude that rubidazone, at these doses and this schedule, appears to be an ineffective agent in these tumor types.

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Chemotherapy for Colorectal Cancer With 5-Fluorouracil, Cyclophosphamide, and CCNU: Comparison of Oral and Continuous Iv Administration of 5-Fluorouracil[1,2]

Brief Reports and Preliminary Communications

Agop Y. Bedikian,* Richard Staab, Robert Livingston, Manuel Valdivieso, Michael A. Burgess, and Gerald P. Bodey[3]

SUMMARY

Forty-six adult patients with colorectal cancer were treated with cyclophosphamide and CCNU administered orally and 5-fluorouracil (5-FU) administered either orally or by continuous iv infusion (FCC-CIF), depending on the availability of hospital beds. The overall response rate in 37 patients with measurable disease was 25%. The response was greater in patients who had had no prior treatment (seven of 20 versus two of 17) and in patients treated with FCC-CIF (six of 19 versus three of 18). Among patients receiving FCC-CIF, response was also greater in those who had received no prior therapy (five of nine versus one of ten). The overall median survival was 7.5 months, regardless of the route of administration of 5-FU. Further investigations are indicated to study the efficacy of 5-FU administered by continuous iv infusion in combination with other agents in patients who have had no prior treatment.

[Cancer Treat Rep 62:1603–1605, 1978]

The results of present chemotherapy for metastatic colorectal carcinoma have been poor. The fluorinated pyrimidine 5-fluorouracil (5-FU) (1,2) and the nitrosoureas (3) are the commonly used antitumor agents with activity against colorectal carcinomas. The superiority of the fluoropyrimidine nitrosourea combinations has been shown (4). Cyclophosphamide given in high, intermittent doses has also demonstrated activity against enteric adenocarcinoma (5). In addition, cyclophosphamide has shown synergism with the nitrosoureas in animal tumor systems (6). Accordingly, a broad phase II evaluation of the three-drug combination of 5-FU, cyclophosphamide, and CCNU (FCC regimen) was initiated in 1974. In this report, we will analyze the results of patients with metastatic colorectal carcinoma treated with the above combination therapy.

PATIENTS AND METHODS

Forty-six consecutive adult patients with histologically proven metastatic colorectal cancer were treated with the FCC regimen every 4 weeks. At the time of registration, the patients were assigned to either inpatient or outpatient treatment groups, depending on their clinical status and the availability of a hospital bed. The 24 inpatients received 5-FU at a dose of 800 mg/m^2/day × 5 days as a continuous iv infusion (FCC-CIF), whereas the 22 outpatients received 5-FU orally (FCC-POF) at a dose of 400 mg/m^2/day in four divided daily doses × 5 days. All patients received cyclophosphamide and CCNU orally at a dose of 200 mg/m^2/day × 4 days and 40 mg/m^2/day × 2 days, respectively. There was no marked difference in the age and sex distribution of patients in the two treatment groups. Nineteen patients had received prior treatment, mostly with 5-FU.

Dose modification, both escalation and de-escalation, was included in the treatment regimen to maintain a tolerable degree of hematologic (absolute neutrophil counts of 750–1000/mm^3 and platelet counts of 75,000–100,000/mm^3) and nonhematologic toxicity. Patients were considered to have had an adequate trial if they were in the study for at least 14 days. The antitumor responses to treatment were assessed in patients with measurable disease ac-

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[3] Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

**Reprint requests to:* Dr. Agop Bedikian, Department of Developmental Therapeutics, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, Tex 77030.

TABLE 1.—Response of patients with measurable disease

Regimen	No. of patients	Response			
		Complete	Partial	Stable disease	Progressive disease
FCC-POF					
No prior 5-FU	11	—	2	5	4
Prior 5-FU	7	—	1	3	3
FCC-CIF					
No prior 5-FU	9	1	4	3	1
Prior 5-FU	10	—	1	5	4
Overall totals	37	1	8	16	12

cording to the following criteria. Complete response was defined as the disappearance of all evidence of disease. Partial response was defined as a > 50% reduction of the sum of the product of all measurable lesions without the appearance of new lesions. If hepatomegaly was chosen as the followable lesion, a response was defined as a decrease of > 30% in the sum of the measurements below the xiphoid process and both costal margins at the midclavicular line. Stable disease was defined as a < 50% increase or decrease in measurable disease for at least 8 weeks without the appearance of new lesions. Progressive disease was defined as a > 50% increase in measurable disease or the appearance of new lesions.

All patients had complete evaluations including history and physical examinations, cbc counts, urinalysis, and determinations of SMA-100 prior to the start of chemotherapy. Ongoing analyses included twice weekly cbc counts, weekly renal and liver function tests, tumor measurements, and plasma carcinoembryonic antigen level determinations at 3-week intervals and appropriate radiologic and isotopic examinations at 2–3-month intervals. All side effects associated with the administration of the treatment were recorded and analyzed. In accordance with institutional policies, a signed consent was obtained from all the patients prior to initiation of chemotherapy.

RESULTS

Seventeen of 37 patients with measurable disease (and thus considered evaluable for response) had received 5-FU prior to entry into FCC programs. Nine patients were not evaluable for treatment response: one because of early death and eight because of difficulty in measuring the areas of tumor involvement. Nine of 37 evaluable patients responded (table 1). The response rate was best for patients with no prior treatment who were treated with FCC-CIF. Disease stabilization occurred in 16 of 37 patients with measurable disease and six of eight patients whose disease could not be measured. The median duration of responses for the two treatment groups combined was 5 months (range, 2–11 months). Responses were seen in five of 24 patients with liver metastases and in three of 12 patients with pulmonary metastases. Responses in the liver were more common with the FCC-CIF than with the FCC-POF regimen (four of 13 versus one of 11 patients). Regressions were also seen in two of three patients with lymph node involvement and in two of four patients with measurable abdominal metastases.

The median survival of all patients from initiation of FCC chemotherapy was 7.5 months. There was no significant difference in survival time between those patients treated with 5-FU orally and those receiving the drug iv (median, 7 versus 7.5 months). Also, the difference in survival time between patients with and without prior exposure to 5-FU was not significant (median, 5.5 versus 8 months). Irrespective of the route of administration of 5-FU, patients who achieved tumor regression or disease stabilization survived significantly longer than patients who failed to improve (median, 11.0 versus 3.5 months; $P = 0.001$).

Hematologic toxic effects, usually occurring during the third week of each cycle, were moderately severe. Severe neutropenia (< 500 cells/mm^3) and thrombocytopenia (< 50,000 cells/mm^3) occurred in 20% and 13% of the courses, respectively. FCC-CIF courses were more often associated with severe granulocytopenia (28% versus 10%) and thrombocytopenia (22% versus 4%) than the FCC-POF courses. A thrombocytopenic patient receiving the FCC-CIF regimen died of massive gastrointestinal bleeding; he was considered the only treatment-related death. Appropriate dose modifications of the drugs included escalation of the dose of oral 5-FU in about 40% and de-escalation of the dose of iv 5-FU in the same proportion of courses. The dose of

CCNU was reduced in 49% of courses of FCC-POF and in 39% of courses of FCC-CIF. However, prolonged neutropenia and thrombocytopenia were common after three courses of chemotherapy.

Toxic effects other than myelosuppression occurred in 66% of patients during 24% of the courses of chemotherapy. These toxic effects were more common in patients who received FCC-CIF than in those who received FCC-POF (39% versus 10% of the courses, respectively). The gastrointestinal toxic effects were nausea or vomiting and diarrhea, associated with 14% and 6% of FCC-CIF courses, respectively, and nausea, with or without vomiting, in 7% of FCC-POF courses. This incidence of gastrointestinal toxicity is much lower than expected and probably reflects under-reporting. Infectious complications were rare but were more frequent in patients receiving the FCC-CIF regimen.

DISCUSSION

Since the introduction of 5-FU in 1957, controversy has existed regarding its optimum dose, schedule, and route of administration. 5-FU administered by continuous infusion (2) and high-dose cyclophosphamide when given over several days (7) have less myelotoxicity than the same doses administered with conventional schedules. In the FCC program, cyclophosphamide and 5-FU were combined with a myelosuppressive drug, ie, CCNU, taking advantage of the above principles. The overall response rate with the FCC program is only 25%; it is higher for patients who had had no prior treatment and in patients treated with 5-FU administered by continuous infusion. The response rate (five of nine patients) for the group of patients with no prior therapy who received FCC-CIF is markedly better than that for any other group in the study. Responses in hepatic metastases were seen more often in patients receiving FCC-CIF than in patients receiving FCC-POF, a result consistent with recent findings (1) and contrary to reports of other investigators (8). The poor response rate to orally administered 5-FU is probably due to the unpredictable absorption of 5-FU from the gastrointestinal tract (9).

The lack of improvement in the survival time for patients treated with FCC-CIF is mainly because of the nonrandomized nature of the study, which favored the treatment of patients who had a better performance status with FCC-POF. A retrospective analysis of performance status of patients in the study showed that 20 of 22 patients receiving FCC-POF were ambulatory and able to do their normal activity without assistance (Karnofsky scale, class A) whereas 13 of 24 patients receiving FCC-CIF were disabled (Karnofsky scale, class B or C). Despite the poorer performance status of the patients receiving FCC-CIF, their survival time was no less than that for the patients receiving FCC-POF. Our results indicate that further study of the efficacy of 5-FU administered by continuous iv infusion in combination with a nitrosourea and other active agents is warranted in patients with no prior treatment.

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LETTERS

Use of the Myocardial Biopsy to Monitor Cardiotoxicity

I would appreciate the opportunity to respond to Dr. Ferrans' paper "Overview of Cardiac Pathology in Relation to Anthracycline Cardiotoxicity," which appeared in the June 1978 issue of *Cancer Treatment Reports*. On page 961, Dr. Ferrans lists five points which "can be identified as presenting limitations to the usefulness of the myocardial biopsy. . . ." I wish to comment on each point:

1. This statement is incorrect. Myofibrillar dropout is and always has been used as part of our grading system as well as vacuolization. This fact has been well-documented in all of our previous and current publications on this subject (1–5); therefore, I cannot understand this misconception. Fibrosis is indeed deliberately avoided in grading since it is a nonspecific finding (particularly in older patients), is hard to quantitate, and cannot be distinguished from fibrosis due to mediastinal irradiation. This reasoning has also been carefully explained in previous publications (1–5).
2. We agree that there is a sampling error risk, but by examining five Epon blocks per piece from multiple biopsies taken at the same time, we have eliminated grading discrepancies to a statistically acceptable point. The following letter in this issue from Dr. Bristow goes into more detail on this point.
3. We have found *no* difference in the scoring of left and right ventricular biopsies in the anthracycline-treated patients in whom biventricular samples have been examined. Furthermore, we have documented anthracycline-induced morphologic changes in 99 patients from a total of 108 biopsied patients (all but two of the patients with a score of 0 had received < 275 mg/m^2). This strongly suggests that the right ventricular septum in man is a representative site.
4. We agree that biologic variations to anthracycline cardiotoxicity are well-documented in animals and man. Since the biopsy is used to quantitate the degree of morphologic damage, surely this is an asset of the technique and not a limitation.
5. Since the biopsy is the only way to distinguish anthracycline-induced heart failure from that due to other causes, far from being a limitation, this is the strongest point of all in favor of the biopsy.

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Margaret Billingham

Department of Pathology
Stanford University School of Medicine
Stanford, Calif 94305

August 22, 1978

We wish to take issue with some of Dr. Myers' comments published as an epilogue to the recent *Cancer Treatment Reports* symposium on the monitoring of anthracycline cardiotoxicity (1). Dr. Myers states,

> "[Endocardial biopsy] involves using a small sample to determine the severity of an essentially focal process. As might be expected, this results in significant sampling errors. In particular, there are a significant number of patients with congestive heart failure whose biopsies only showed mild (grade 1) damage."

We are curious as to the source of Dr. Myers' information. The Stanford series includes biopsies from 11 patients who were in clinically overt heart failure at the time of or within 6 months of study. Nine of these patients had + 3 (severe) biopsy abnormalities. One patient with a long history of excessive ethanol intake had a + 2 (moderate) biopsy abnormality and a 78-year-old patient with severe mitral regurgitation by catheterization and clinical criteria had only a + 1 (minor) abnormality.

These latter two patients probably had mixed cardiac pathology, with anthracycline-related cell damage superimposed on alcohol-related cardiomyopathy and valvular disease. Of 15 patients with a + 3 biopsy, seven were in overt heart failure at the time of study, two developed heart failure within 6 months (after having their drug stopped because of the biopsy findings), and five had hemodynamic abnormalities at catheterization. One of these five had a + 3 biopsy on an initial study and adriamycin was stopped. When restudied 16 months later, this patient had developed catheterization abnormalities but remained asymptomatic. The final patient had a + 3 biopsy and no catheterization abnormality when studied recently; adriamycin was discontinued and the patient will be re-evaluated in the future.

Evidence for the lack of a significant sampling error comes from patients studied serially. We have biopsied 14 patients twice and three patients three times; every repeat biopsy was either unchanged in pathology score (but usually with slight progression within categories) or had increased in grade, as predicted in a process that is dose- and time-dependent. If there were a sampling error it would be expected that an occasional second or third biopsy would show less cell damage.

Another point against sampling error is that multiple specimens from the same patient are in general agreement; we have not had examples of a severe (+ 3) abnormality in one specimen with little or no change in another.

It is surprising to us that sampling error is not more of a problem since, as Dr. Myers points out, we are dealing with relatively small amounts of tissue. The reason why it is not a problem is probably because, although the process is microscopically focal, no large areas of left ventricle or distal right ventricular septum are spared. Also, we have always taken great technical pains to minimize sampling problems, since at least three separate pieces of tissue are obtained during each study and Dr. Billingham's techniques guarantee that any abnormality present in a biopsy specimen will be analyzed at the ultrastructural level.

The endomyocardial biopsy is the only procedure that can directly assess the anthracycline cardiomyopathic process. Measurements of cardiac function, no matter how sophisticated, will only reflect the response of the heart to this process. Although we consider a careful hemodynamic evaluation (including an exercise study) to be essential to the full characterization of anthracycline cardiomyopathy (2), the information provided is less specific. At present, we would not base dose-limitation decisions on hemodynamic data alone. It remains to be seen if any noninvasive test of cardiac function will be of any value other than as a screening tool for referral to biopsy and catheterization studies.

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Michael R. Bristow
Jay W. Mason
John R. Daniels

Cardiology Division
Stanford University School of Medicine
Stanford, Calif 94305

September 5, 1978

ANNOUNCEMENTS

International Symposium on Gestational Trophoblastic Tumours

London, England, May 23 and 24, 1979

A 2-day symposium on Gestational Trophoblastic Tumours is being held at Charing Cross Hospital, Fulham Palace Road, London, England, on May 23 and 24, 1979. It is planned to cover the epidemiology, immunology, biology, endocrinology, therapy, monitoring, and followup services of these tumours.

Application forms and further information may be obtained from: Dr. E. S. Newlands, Department of Medical Oncology, Charing Cross Hospital, Fulham Palace Rd, London W6, England.

Seminars in Oncology at Roswell Park Memorial Institute

Buffalo, New York

There will be a seminar on "Recent Advances in Gynecologic Oncology" on February 8, 1979, organized by the Department of Gynecology at Roswell Park Memorial Institute. The chairperson is Dr. Joseph Barlow.

There will be a $25.00 registration fee. *For further information contact:* Ms. Claudia Lee, Cancer Control, Roswell Park Memorial Institute, 666 Elm St, Buffalo, NY 14263.

5th Course of Medical Oncology

Lecce, Italy, November 30 to December 2, 1978

The 5th Course of Medical Oncology, organized by the Italian Association on Medical Oncology in collaboration with the General Hospital "V. Fazzi" of Lecce and the Italian League Against Cancer (Lecce Section), will be held in Lecce, Italy, from November 30 to December 2, 1978, at the President Hotel.

The main topics will be: advances in cancer chemotherapy and interdisciplinary treatment of breast cancer, malignant non-Hodgkin's lymphomas and Hodgkin's disease, soft tissue sarcomas, lung cancer, gastrointestinal carcinomas, malignant melanoma, testicular carcinomas, bladder carcinomas, and cerebral tumors. The course will also deal with recent progress in childhood tumors.

The subscription fee is Lit. 85.000 (covering lunch and proceedings of the course). *For further information contact:* Prof. Marcello Filotico, Ospedale Generale Regionale "V. Fazzi," Lecce, Italy.

Funds Available for Cancer and Nutrition Research

The National Cancer Institute's Diet, Nutrition and Cancer Program is interested in receiving grant applications for cancer- and nutrition-related research concerned with basic and applied studies in the areas of etiology, treatment, rehabilitation, and training. It is not the intent of this announcement to make or imply any delimitation relative to the nature or scope of the research which might be proposed.

Applications are also requested in the area of training of individuals in the broad area of diet and nutrition as these relate to cancer. Both individual and institutional support is available through the Institutional Training Grants (T-32), Individual Postdoctoral Fellowships (F-32), and the Cancer Research Career Development Program (RCDA) (K04).

More detailed information can be found in the National Institutes of Health's Guide for Grants and Contracts, vol 7, No. 14, Sept 25, 1978, or by contacting Neil M. Ellison, MD, National Cancer Institute, Bethesda, Md 20014: telephone (301) 496-9326. *The deadline for receipt of applications for FY 1979 funding is December 1, 1978.*

American Board of Internal Medicine Policy Concerning Certification in Two Subspecialties

While not wishing to encourage certification in more than one subspecialty, the Board has recently established training requirements for candidates desiring admission to two subspecialty examinations, particularly in Hematology and Medical Oncology. All such candidates should direct written inquiries to the Board detailing their training. For candidates who undertook their first year of subspecialty training during the academic year 1974–1975 or after, the minimum training required for dual certification is 3 years divided between the two subspecialties with at least 1 year of clinical training in each. Beginning in 1978, candidates planning for dual certification must apply for the examination in which initial subspecialty certification is desired and must become certified in that subspecialty before being permitted to undertake an examination in the second subspecialty. If unsuccessful in certification in the initial subspecialty, candidates will only be admitted to the examination in a second subspecialty if they also meet the full training requirements (currently 2 years) for that subspecialty.

This clarification of Board policy requires declaration by candidates of what might be called their primary subspecialty. It applies only to those candidates who have had < 2 full years of training in each of the two subspecialties.

Fourth Annual "Symposium on Advances in Cancer Treatment Research"

Baltimore, Maryland, April 19–21, 1979

The Baltimore Cancer Research Center, National Cancer Institute, and the Program of Continuing Education of the University of Maryland School of Medicine are pleased to announce their 4th Annual "Symposium on Advances in Cancer Treatment Research," to be conducted in Baltimore, Maryland, April 19–21, 1979, at the Friendship International Hotel.

An experienced and internationally recognized faculty will participate. This Symposium is designed to update physicians and surgeons on some of the most recent developments in cancer treatment and current research including areas of clinical management, etiology, and cellular biology. Faculty members will be available throughout the Symposium to discuss any questions or interests of individual course registrants. A visit to the Baltimore Cancer Research Center can be scheduled for interested persons. The course is accredited for 16 hours of Category I credit for the PRA of the AMA by the Program of Continuing Education, University of Maryland School of Medicine.

***For further information please contact:* Jack Mason, PhD, Assistant Dean for Continuing Medical Education, 10 S Pine St, Rm 300E, Baltimore, Md 21201 (phone: 301-528-3956).**

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